THE
AMERICAN NATURALIST

VOL. LXXI *September–October, 1937* No. 736

CAN ARTIFICIAL SELECTION PRODUCE UNLIMITED CHANGE?

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THE question of the amount of change in a character which can be accomplished by selection has been debated for many years, opinion fluctuating now in one direction, now in another. It is obvious, of course, that the nature of the character under selection, or of the case, may establish limits to the amount of change. The part can not be greater than the whole—an eye can not be larger than the body of which it is a part. Nor can that which is complete be made more complete—a sugar beet can never contain more than one hundred per cent. sugar. There are, also, other instances in which selection encounters limits to its effectiveness which arise from the nature of the case itself, such as, for example, a limit to the speed of the race horse. But aside from limits of this kind, the idea of a limit established by the nature of the mechanism of inheritance has arisen as part of the development of modern genetics. This conception arises because the gene has been shown to be a stable unit, change in a gene occurring only at very long intervals. A species or stock is believed, therefore, to possess a complement of genes, varying combinations of which cause the differences occurring among the individuals comprising the species or stock. The complement of genes is regarded as a substantially fixed quantity, which therefore establishes barriers beyond which it is thought that change can not be extended.

Hence, the view has arisen that selection is powerless to change a character beyond an amount which is determined in advance by the genetic constitution of the species or stock under selection, even though the nature of the character concerned, such as, for example, body size in animals, offers opportunity for large change. Selection, without the help of hybridization, is not regarded as an efficient agent for the improvement of agricultural plants and animals, since the amount of change in a character which it can effect is believed to be small. A recent expression of the modern view on this point is found in Jennings's (1935) statement: "But after this (selection) has continued for a number of generations, improvement ceases; no further progress is made. Selection ceases to be effective."

The question of the amount of change which can be accomplished by selection is of great importance to every one because of its bearing on continued improvement of farm crops and live stock. Moreover, if, as seems implied in Jennings's statement, no large amount of change is possible under selection, even if this amount is increased through hybridization, the fact of limit is not abolished. Now, Jennings's statement is based partly on theory and partly on experimentation, but of the many experiments on selection which have been made only a few have been concerned with characters which are not subject to what are often called "physiological limitations"; *i.e.*, limits which arise from causes other than the genetic nature of the organism. In the case of the few exceptions, it is not clear that the methods of selection employed were well adapted for bringing out all possibilities or that too much haste was avoided in concluding that further progress was impossible.

The theory on which the conclusion is reached—that selection is able to produce only a limited amount of change—is the multiple factor theory which states that genetic variation is due to recombination of genes and that complete homozygosis establishes limits to the

amount of possible change. Now, the nature of the multiple factor hypothesis is such that it is very difficult to prove that all genes necessary for the amount of change actually observed under selection were not in the chromosomes of those individuals with which an experiment begins. There are, however, two experiments which point to the possibility of accomplishing an amount of change by selection which transcends the amount expected from an original complement of genes and indicates that present ideas of limits are much too narrow. These two instances are interesting in another respect, because they concern characters which seemingly offer opportunity for little, if any, modification. The amount of change which has been accomplished in these characters demonstrates that it is not possible to predict in advance the extent to which selection may be effective. They could be presented as examples where the limit of change has not been reached, without mention of the hereditary mechanism involved. However, inasmuch as the multiple factor theory can not be applied to either of these cases without assumptions that need to be investigated experimentally, the nature of the difficulties encountered by the multiple factor theory will be indicated without going into the problem too deeply at this time.

While selection experiments may deal with qualitative or quantitative characters, they are concerned, ordinarily, with a variable quantitative character, the variability of which may be expressed by a curve showing the extent of variation and the proportions of the population occurring in each of the several classes. With this kind of material, it has been demonstrated repeatedly that selection produces marked change, as, for example, in the classical case of Castle's hooded rats. The changes that occur under selection are said to be the result of the sorting out of genes already in existence in the population with which the selection began. In some instances, notably Bar Eye in Drosophila, the location on the chromosomes of some of the genes influencing the number of facets in the eye has

been determined. These experiments are familiar to all geneticists and need no review here. For these experiments, the multiple factor theory is both satisfactory and adequate. It provides, moreover, sufficient basis for the belief that much worth-while improvement in the useful qualities of farm animals could be accomplished by selection, and so much use has been made of the multiple factor theory at Mount Hope Farm, always in the earlier years with the thought that, after a comparatively small number of generations, further improvement in the character under selection would prove to be impossible. But as advance after advance was made, the limit of improvement in sight retreated, as the horizon retreats before the traveler, bringing with it first questions as to the point at which progress would cease and finally the realization that the amount of possible improvement, if not unlimited in fact, was at least so indefinite that for practical purposes the characters on whose improvement we are engaged can be treated as if selection could, with many characters, cause practically unlimited change, as Charles Darwin and his earlier followers apparently believed.

The theory of multiple factor inheritance of quantitative characters was proposed by Nilsson-Ehle, on the basis of duplicate genes for color of wheat, and refers variation in the character under examination to activities of genes distributed according to the rules of Mendelian segregation. The theory has been tested in several ways and, because these tests are favorable to it, it has been generally accepted as the correct explanation of genetic variation in quantitative characters and of the changes in such a character which occur under the aegis of selection. Although the theory is commonly stated in a comparatively simple form, there is no reason why all modifications in behavior of genes should not apply to it. Moreover, the number of genes concerned may be increased or decreased to fit the needs of any situation. Hence, in a given situation, if the amount of change accomplished by selection is more than anticipated, the breeder is always at liberty

to explain his results by increasing the number of genes present in the stock with which he begins selection or to resort to any known or conceivable modification of gene activity.

The multiple factor hypothesis is open to a number of tests other than that commonly used. But the application of these tests requires so large an expenditure of time and effort that they are rarely, if ever, employed. We will, therefore, omit discussion of these tests and pass at once to a consideration of the two experiments which clearly indicate unexpected possibilities in selection.

The first of the two selection experiments, made on material which in the beginning appeared most unpromising for producing considerable change in the character to be modified, is an experiment in increasing the number of bristles on the scutellum of *Drosophila melanogaster,* made by Fernandus Payne and published in 1918. Although this experiment is of very great significance for what is often called the "selection problem" because of the manner in which it was conducted—as described later—it has received very little attention from geneticists.

Papne began his experiment by counting the bristles on the scutella of 2,861 Drosophila. The result is shown in Fig. 1 by the vertical line and the two small humps on either side of this line. In this population, the number of scutellar bristles varied between three and five, 99.5 per cent. of all individuals having four bristles only. Variability is, therefore, very low, which may be taken as evidence that the number of heterozygous genes affecting bristle number is small. (A possible exception to this statement is discussed beyond, in connection with another experiment which will be described in due course.) But this is not the only evidence of a small number of heterozygous genes influencing bristle number. Selection for larger number of bristles was commenced with a five-bristled female and her four-bristled brother. These two individuals, therefore, carried all the genes—mutations excepted—homozygous as well as heterozygous, that fu-

ture generations of their descendants could possess. Since genetic variation among the descendants of the two foundation individuals requires the presence of one recessive gene, at least, out of four at several or many loci in the chromosomes of the two foundation individuals, the recombinations occurring in F_1 and F_2 should provide a satisfactory index of the potential variation to be expected from this source.

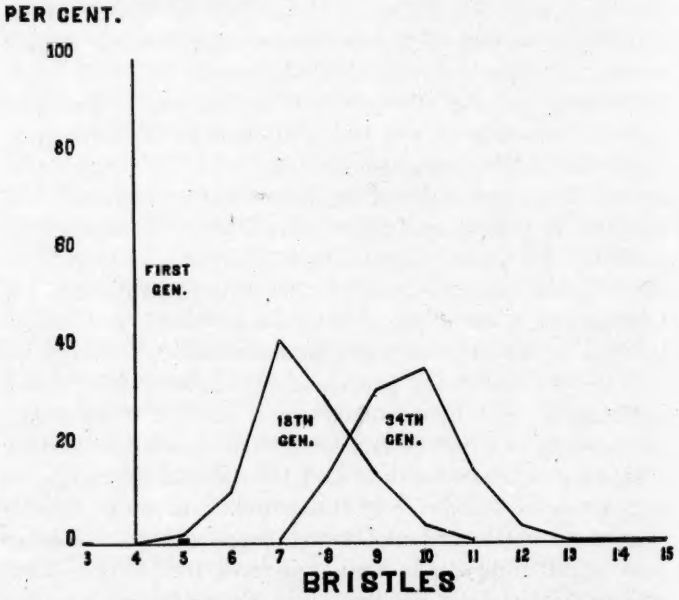

FIG. 1. Curves of variation in number of bristles on the scutellum of *Drosophila melanogaster*, plotted from data in Payne's Table 1.

The mating of the foundation pair produced 228 offspring—226 had four bristles each and 2 had five bristles each. The next generation was produced by mating fourteen pairs of these offspring, brother to sister. The offspring of these fourteen pairs, constituting the F_2 generation, should reveal the degree of variation due to heterozygosis not revealed in F_1, provided the number of F_2 individuals is sufficiently large. 3,625 offspring

resulted; 3,523 had four bristles; 90 had five bristles and 12 had six; or in percentages, 97, 2.5 and .3 of one per cent., which is satisfactory evidence that not many pairs of heterozygous genes were free to influence bristle number. Mating mostly brother to sister, Payne then selected stringently for extra bristles for eighteen generations and less stringently for twenty generations more, the results of which are shown in Figs. 1 and 2. Since both the F_1 and F_2 generations gave little evidence of heterozygous genes, and *since the extra bristles developed by selection are to a large extent dominant over the normal number,* Payne should have found it impossible to increase bristle number as he did by rearranging the genes already present in the two individuals with which he began the experiment, unless one is willing to explain the results of his experiment by assigning properties to the genes involved—which genes are not now known to possess, or by putting into the explanation the very conditions or circumstances which it is sought to explain—and then explain Payne's results in terms of these properties or circumstances. Moreover, beginning with a race of low variability and mating brother to sister, which leads theoretically to increasing homozygosity and decreasing variability, Payne increased variability and created something of which there was no evidence in the population with which he began his work.

It is very hard to believe that the genes for extra bristles were hidden in the chromosomes of the two flies with which the experiment began. But, if they were not there, they must have arisen through mutation—unless one is willing to believe that selection in some way brought about the change. Payne reached the conclusion that two, or possibly three, mutations occurred which, at the time he wrote, was a reasonable conclusion. Moreover, by means of linkage tests, he was able to show that changes had occurred in the first and second chromosomes. However, there are a number of reasons why Payne's success in increasing bristle number can not be attributed to muta-

tions. As every one knows, mutations of the kind which Payne evidently had in mind—mutations at definite loci in the chromosomes—are rare and erratic happenings. It is therefore, a most remarkable circumstance in the light of what is known to-day about rate of mutation in Drosophila that exactly the right kind of mutations should have occurred when they were most needed. The contradiction between mutation rates in Drosophila known to-day and the rate of progress made by Payne are shown by the following facts. Sinnott and Dunn (1932) page 174 *ff.*, state that "some twenty-five to thirty million of individuals have been bred under observation, and about five hundred gene mutations have been identified." This is a rate for all kinds of mutations of one in fifty or sixty thousand flies. Since scutellar bristles were counted on 50,425 flies, only one mutation of any sort was to be expected. If, now, the figures for particular mutations, reproduced by Sinnott and Dunn from Timoféef-Ressovsky, are examined, it is found that the most frequently observed mutations, white and notch, have occurred about once in a million individuals. The observed rate of spontaneous mutations, therefore, is much too low to account for the numerous small increases in number of bristles that occurred. Besides, it is a most interesting circumstance that the one mutation expected on the basis of the number of flies studied actually occurred and that it affected bristle number, but ironically enough, it was a mutation exactly opposite the direction of selection, the mutation Payne called "reduced."

It is clear from Payne's account of these experiments that he regards the mutations, which he thinks occurred, as mutations which may be designated as "release" mutations; *i.e.*, mutations in genes which hitherto had prevented other genes from influencing bristle number—just as a mutation in the polled gene in cattle to horned releases all the genes influencing shape, color and size of horns. But this kind of mutation should reveal itself, not by a gradual increase in bristle number, such as is

shown in the records, but by the appearance of a sudden increase in variation among the offspring of particular pairs of flies, the exact amount of variation which appears being determined, of course, by the genetic constitution of the affected individuals. Of this, there is no record.

Inasmuch as Payne identified the points in his experiments, at which each mutation is supposed to have occurred, both by statements in the text and by diagram, attention should be directed to the parallelism that exists throughout Payne's experiment between the average number of bristles in each generation of parent flies and the average number in the corresponding generation of offspring. An examination of Fig. 2, which plots these figures, shows that the points, Ms, which are identified as

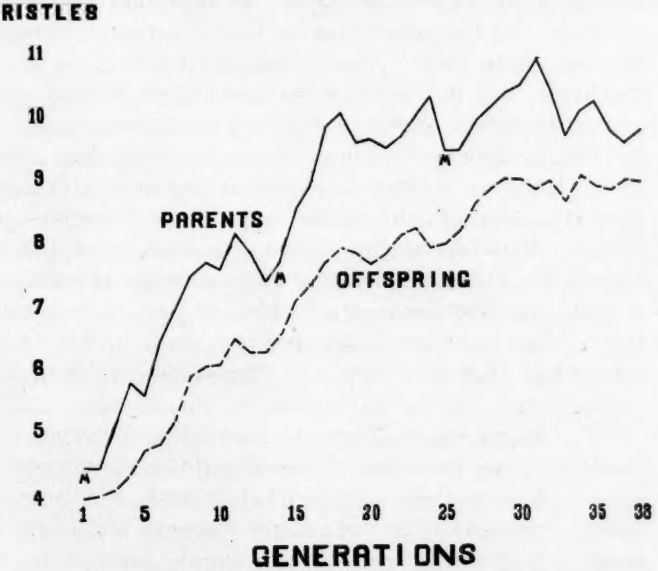

FIG. 2. Comparison of average number of bristles for each generation of parents and offspring. This figure is like Payne's Fig. 2, except that the lines of interpretation inserted by Payne are omitted. An "M" marks the approximate position of the points at which mutations were supposed to have occurred. "When a new mutation occurs, the curve goes steadily up until the race is homozygous for this factor." Payne '18, page 15.

marking the appearance of a mutation, correspond to temporary declines in parental averages. Moreover, a comparison of the bristle number of the flies available for breeding in each generation with the average recorded for the parents actually used, shows a lower number than is to be expected if those flies having the largest number of bristles in each generation had become the parents of the next. Hence, it is highly probable that the fluctuations in average number of bristles among the offspring represent fluctuations in the stringency with which the parental flies were selected, and that the correspondence in the fluctuations of parental and offspring averages is due to corresponding resemblances in inheritance. Although this correspondence is apparent throughout the whole course of the experiment, there is one place of special interest. An examination of the line of parental averages reproduced in Fig. 2 shows, beginning with generation seventeen, that the parental average varied on both sides of ten throughout the remainder of the experiment. At the twenty-fifth generation, the parental average stood at 9.55 bristles. It was the same the next generation, and then it increased, during the next five generations, to eleven. This increase in parental mean is accompanied by a rise in average number of bristles in the offspring of slightly less than one, a result which indicates very clearly that further increase in bristle number might have been made, had that end been the immediate object of the experiment.

This experiment of Payne, in increasing the number of bristles on the scutellum of *Drosophila melanogaster,* is clear evidence that the breeder need not hesitate to attempt the improvement of any character which in his judgment needs improvement, even though slight variation in the character seems to offer little opportunity for improvement. Payne's success in increasing bristle number shows that the necessary variation may, sometimes at least, arise in such close association with selection that it is hard to escape the conclusion that selection is an

indispensable agent or accessory in producing the variation. This fact can not be denied, even if it can not be explained satisfactorily, either by mutations at definite loci or by genes concealed in the two individuals with which the experiment was begun.

The second experiment, which demonstrates that selection is able to accomplish large changes in a character which seemingly offers little opportunity for improvement, begins with the appearance of a mouse in the colony at Mount Hope Farm which had a few white hairs on its forehead. This is very likely an independent occurrence of the condition called "head-dot" by Keeler (1935). Because it was thought that this mouse might provide material for the study of breeding problems, he was mated to relatives, in the hope that white hairs on the forehead would prove to be an inherited character. Among the offspring of this mating were other mice with a few (1 to 20) white hairs on their foreheads. Selection for larger numbers of white hairs was immediately effective, mice appearing having a slightly larger—but still small—number of white hairs on their foreheads. At this point in the experiment, a number of mice representing the condition of the character as it then existed were set aside and allowed to breed without further selection, by the expedient of removing the older mice when the cages became too crowded. Selection for larger spots was continued with the remainder of the stock. At the present time, after some seventeen generations of selection, these two lines are very different in appearance. Among the mice of the unselected line are mice without a single white hair on the forehead, while at the other extreme in this line are mice with a small white spot covering an area of approximately six square millimeters (Fig. 3, C). In the selected stock, the number of white hairs has steadily increased until on those having the largest number, the white area exceeds 160 square millimeters (Fig. 3, D and E). Mice with much smaller white areas occur, but the smallest are no longer as small as the largest of the unselected line.

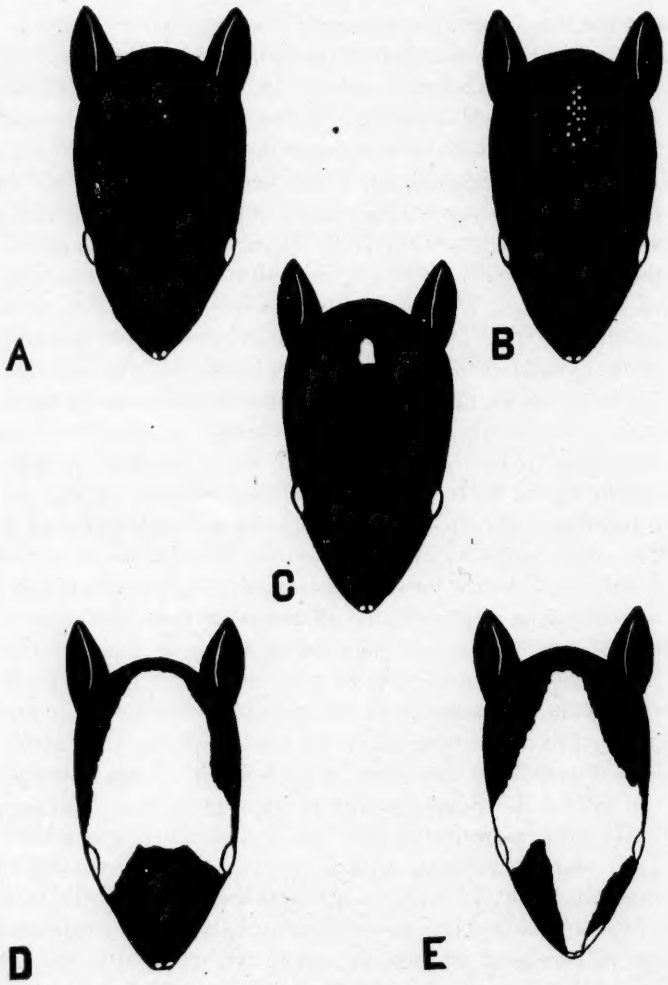

FIG. 3. Diagrammatic representation of the anterior surfaces of several adult mice to show differences in the extent of the skin areas which produce white hairs. Figs. A, B and C are from the unselected line. In A and B, the points of outgrowth of white hairs are represented by white circles. Fig. C represents one of the larger white areas that occurs in this line. Figs. D and E represent the largest solid white areas that have appeared to date in the selected line, after seventeen generations of selection. Measurements, made on a living adult mouse, with a white skin area slightly less in extent than that shown in E, give the area as 165 square millimeters, which may be contrasted with the white area in Fig. C, which covers only 3.1 square millimeters.

Moreover, as the experiment proceeds, it is becoming increasingly clear that the smaller sizes of spot in the selected line disappear, one by one.

The history of the mouse experiment is the kind of history that can be written of any selection experiment, except that it begins with a small, insignificant character, so small that most people fail to recognize it until it is pointed out to them. This experiment is significant in another way because, when the pedigrees are examined, it is found that all later generations are descended from five individuals. It is to be supposed, therefore, that the genes for large areas of white, which developed under selection, were distributed among the chromosomes of the five foundation animals as is required by the multiple factor hypothesis, but that conditions in the five parent mice were such that these genes had no opportunity of expressing themselves. The conditions that prevented the genes from manifesting themselves in the five foundation mice evidently persist in the unselected line, because no increase in size of the white area has occurred in it. Now it is clear that the genetic variation in the size of the white area in this line can not be due to genes homozygous for the same alleles in all five foundation animals. To have genetic variation, heretozygous genes are necessary, and heterozygous genes under random mating become distributed according to the rules of Mendelian segregation, modified by linkage, and form a curve of variation whose shape and extent depend upon the number, frequency, dominance, etc., of the various genes. While studies of curves of variation do not permit of entirely satisfactory conclusions concerning the number and character of the genes involved in producing a given curve, nevertheless, such studies afford information which permits of some useful conclusions. Now, in the unselected line, the range of variation extends from less than zero[1] at one extreme

[1] The sub-zero condition is recognized most easily in mice that may be called black, although they differ in several respects from self-black mice. In the sub-zero black mice, the black is a shade or two less black than that

to a small spot at the other, whose area, so far as present observations go, does not exceed six square millimeters. The mode has not, as yet, been determined exactly, but probably lies between ten and twenty white hairs. This curve, therefore, presents no evidence of arising from such a number of pairs of heterozygous genes as is required to explain the appearance of the large spots that have been slowly developed by selection, which at their maximum are now twenty-five times the size of the largest spots that appear spontaneously in the unselected line.

Since the unselected line is descended from the same five foundation mice as the selected line, and since phenotypically it remains in the same condition as the early generations of offspring of these five mice, the question arises: How are the genes controlling the size of the white area to be regarded? On the multiple factor theory, the genes for the large white area developed by selection must—unless lost by inbreeding or natural selection—exist in the unselected line. They must, therefore, be numerous because if they were few in number, they should by recombination produce mice with as large white areas on their foreheads as occur in the selected line, but this has not happened. However, there are certain possibilities which may modify this statement. The genes for large area may have existed with low frequency in the foundation mice, say one in ten, which is the lowest frequency possible with only five foundation mice; or plus genes may have been linked with zero or minus genes, or they may have been linked with lethals; or these genes may have existed in a series, each having slightly more ability in extending the white area than its predecessors, but each being recessive to an allele of slightly greater inhibiting power; or in a series of multiple alleles. Selection could

of ordinary black mice, the muzzle and nose are lighter colored, and one or more toes or parts of a toe may be white, while the tail often has one or more light-colored areas. Although these sub-zero mice superficially appear to be selfs, it is clear that they lack the allele for self and, therefore, are not self mice of the sort usually described under this term.

produce the observed results on any of these assumptions, although the mechanism is more or less different in each case, but it is not necessary to enter into a discussion of the differences at this time.

An explanation of the increase in size of the white spot on the head of the mice is first to be sought in multiple factors; *i.e.*, genes once distributed among the 200 chromosomes of the five foundation animals but which have been brought together in the selected stock, so that each individual now has sufficient genes to produce the larger sized spots. The genes, instead of being scattered among individuals as they were in the foundation animals or their unselected descendants, are concentrated to a greater or less extent in each individual of the selected stock. If the effectiveness of selection in this case is due to concentration of genes, then when a cross is made to mice that do not carry these genes, the genes that have been brought together should segregate in F_2 and the work of selection thrown back toward the middle ground between the selected and the unselected races. For determining if this has happened, the triangle method of crossing is well suited, as shown in Fig. 4.

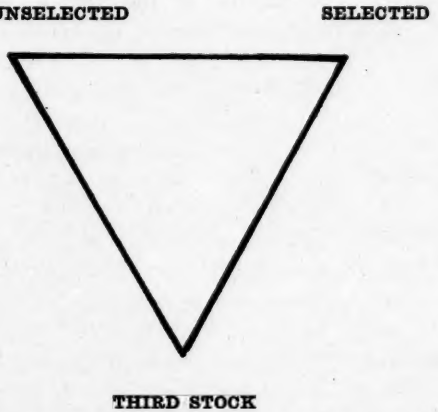

FIG. 4. Diagram to illustrate a triangulation, the data for which are given in Table 1.

In this method, the unselected stock and the selected stock are each mated with a third stock, as well as with each other. The mating of the unselected stock with the third stock is needed to show that the third stock does not carry genes for the larger sizes of spot. By making the three crosses shown by the diagram, Fig. 4, the presence or absence in the third stock of this kind of gene can be demonstrated, because if they are present to the same extent as in the selected stock, the cross of unselected to the third stock is the same in this respect as the cross between the unselected and selected stocks, while if they are no different from the unselected stock, then the mating of selected stock with the third stock is the same as the mating of unselected with selected stock. This line of reasoning can be applied to any concentration of modifying genes.

In making this test, it is clear that spotted mice can not be used for third stock. Hence the only stocks suitable for use as third stock are mice homozygous for self-coloring, which makes it necessary to carry the experiment to the second generation. Three triangulations of this kind, on which statistics are being accumulated, have been undertaken. The nature of the results are shown in Table 1. The third stock used in the triangulation shown

TABLE 1

Crosses of Unselected and Selected Line Mice with Agouti and with Each Other

Mating	Generation	Classification of offspring											Totals
		Number of white hairs		Size of spot (Arbitrary grades)									
		0	Few*	S†	1	2	3	4	5	6	7	8	9	
Unselected by Agouti	F_1	62	8	1										71
	F_2	79	28	5	1	1								114
Selected by Agouti	F_1	15	55	4	18									92
	F_2	55	84		16	13	3	20	7	12	3	1	2	216
Unselected by Selected	F_1				6	5	7	5	6	7	1	0	1	38
	F_2			7	8	7	14	17	23	35	21	3		135

* One to twenty white hairs. The number of white hairs were not counted in part of the F_2 individuals but were recorded merely by the word "Few." Compare Table 2.

† S = Small spot, intermediate between the grade represented by somewhat more than twenty white hairs (see Table 2) and Grade 1.

in Table 1 were agoutis inbred for twenty-four generations by Dr. Leonell C. Strong before coming into our colony several years ago, and so are presumably nearly or completely homozygous. The unselected line mated to agoutis produced an F_1 which is mostly self-colored, but an occasional individual had a few white hairs on its forehead and one had a small spot. F_2 conforms to the expectation of a mono-hybrid, three fourths being self and one fourth having a few white hairs on their foreheads. Exceptionally, as in the unselected stock itself, an individual with a small spot appears. This result agrees with Keeler's (1935) observations.

Consider next the results of crossing the selected line with the agoutis. It is apparent that F_1, Table 1, is not like the F_1 of the previous mating, but differs in that the mice usually have white hairs on their foreheads, in some cases in sufficient number to form a conspicuous, if small, white spot. Turning now to the F_2 generation, it appears that three classes can be recognized; first, self, which number about one fourth of the total; second, mice with a few white hairs or a small spot, numbering about one half of the total; third, and grading into the second class, a group of mice with spots of various sizes which correspond in general with the variation in spot size of the selected line at the time these matings were made. This group numbers about one fourth of the total. Conspicuous among the mice of this group are two individuals in the total of seventy-seven, which exhibit a spot as large as the largest of the selected stock at the time the cross was made.

The third mating is that of the selected with the unselected race. Here, the F_1 consists of mice with spots covering the range in spot size of the selected stock. F_2 is similar to F_1.

The figures given in Table 1 for the F_1's are compiled from the records of individual females, but the F_2 figures result from mass matings secured by mating all the males of a litter with their sisters. Sometimes, litters from

more than one female have been put into one cage. While figures have been secured rapidly by this means, they are, to some extent, misleading because of individual differences in genetic constitution. Thus B295 of the selected line, mated to an agouti male, in two litters produced 8 self mice and 10 mice with one to five white hairs, while 7 other females of the selected line, mated to the same male, in one litter each, produced 7 self, 14 with one to five white hairs, 14 with five to fifteen white hairs, 7 with more than fifteen and 11 with small spots. Thus, it is apparent that while the lumped figures present the broad outlines of the results, there are details that require further investigation. Indeed, as the selection experiment continues, it seems inevitable that extensive revisions in the data for all matings in which the selected stock is used will be necessary.

Further important data on the influence of single individuals on the character of the records are shown in Table

TABLE 2

MATINGS OF TWO SELECTED LINE MALES WITH THREE AGOUTI FEMALES

Dam's No.	Sire's No.	Sire's grade	Classification of offspring F_{1s}																Totals
			Number of hairs							Size of spot (Arbitrary grades)									
			0	1–5	6–10	11–15	16–20	20+	S*	1	2	2	4	5	6	7	8	9	
177	213	9			2	2				3									7
176	213	9		1	1				2	2									6
223	248	9		2	1				2	2									7
	Total: F_{1s}			3	4	2			4	7									20
			F_{2s}																
			1–20+ combined																
177				6		33				3	4	2	3	1	5	3	0	1	61
176 & 223†				19		23				12	4	1	5	5	6	0	1	1	77
	Total: F_{2s}			25		56				15	8	3	8	6	11	3	1	2	138
						71 (Inside F_1 range)				42 (Outside F_1 range)									

* Small spot, intermediate between twenty white hairs and grade one.
† *i.e.*, Their F_1 offspring were combined in a single breeding cage.

2, in which the results of mating three agouti females to two males of the selected line are given by themselves in order that they may be compared with the lumped figures given in Table 1. Here, the entire F_2 generation consists of 216 individuals divided into 55 self, 84 few and 77 spot,

while the F_2 from the two selected males, Table 2, consists of 25 self, 56 few and 57 spot. The difference between these two sets of figures is 30 self, 28 few and 20 spot, which are the offspring of the F_1 mice not considered in Table 2. It is also important to note that of the 18 F_2 mice of grade six or higher, Table 1, all but one animal of grade six are from the matings shown in Table 2. Now, since some of the F_1 mice fall in grade one, the segregates are represented by only 42 mice, of which 17 are high grade and 2 reach the maximum phenotype of the selected stock as it existed at the time the cross was made. Thus, it is evident that much depends upon the individuals used in particular matings. Indeed, the matings already made suggest that the upper limit of size of spot which can be recovered in F_2 in matings of this kind is directly related to the size of spot of the grandparent used in the matings.

Two conclusions can be drawn from this experiment. First, the third stock, the agoutis, do not carry concentrations of genes for large size of spot, since the mating of unselected with this stock produced in F_2 only mice like the unselected race.

Second, since the agouti race does not carry a concentration of genes for large spot, and since the third group—those called "spot"—of the F_2 of this cross is like the selected stock, and since the range of variation in the size of the spots of this group is substantially the same as the range of variation of the selected race itself, it is highly improbable that the large spot, developed by selection, owes its existence to an accumulation of genes which were distributed among the one hundred pairs of chromosomes of the five foundation mice, because if the genes for large size of spot were distributed at the beginning of the experiment among all or even several of the chromosomes, selection would have gathered the chromosomes bearing these favorable genes into the individuals represented by the selected stock. Now, if it is assumed that the most favorable concentration of chromosomes is represented by males 213 and 248, Table 2, both of grade 9, it is to be expected

that, when these mice are mated with agoutis, segregation would occur in F_2 and the concentration that has been built up over the generations of selection would be lost. But the contrary has occurred and the group called "spot," instead of consisting of mice with small spots, contains a large proportion of mice having the larger sized spots. Indeed, this group practically duplicates the selected lines. Even the extremes are recovered in what is numerically a small group. Moreover, if one is inclined to think that the recovery of the large spots in F_2 represents the action of a few genes, he is confronted with the problem of determining how these genes were arranged in the chromosomes of the five foundation mice so that they remain concealed in the unselected line as well as in the foundation mice. Thus the theory of numerous freely segregating multiple factors appears not to apply in this case. However, the possibility that some modification of the multiple factor hypothesis can be developed remains.

According to modern genetic theory, the genes for large areas of white on the forehead existed in some form in the chromosomes of the five foundation mice. Therefore, it is necessary to consider other possibilities than the one just discussed, but of all that have been considered, only one seems at all applicable to this case. This explanation assumes that in the foundation animals, a series of alternating, but sometimes staggered, plus and minus (or zero) genes were located in one pair (possibly more) of chromosomes and that the minus genes almost balanced the plus genes, thus giving rise in the unselected stock to the limited areas described above. The fact that the size of the spot increases under the pressure of selection is referred to the accumulation of cross-over classes containing more plus than minus genes. The accumulation of plus genes is supposed to occur in this way. If, in the beginning, there is an equal number of plus and minus genes, alternating one with the other, but occasionally staggered so that plus genes of one chromosome are paired with minus genes of the other, cross-overs may occur in such a way that one

chromosome after synapsis has more plus genes than the other. The individual which receives this chromosome exhibits a slight increase in the size of the spot and, being selected as a parent of the next generation, passes the chromosome containing more plus genes to part, at least, of its offspring. When these mate, it happens that some of their offspring receive two such chromosomes. The stock of such individuals is increased by multiplication, and in one or more of these, a further increase of plus genes occurs in one or more individuals through another cross-over. This process, repeated over a sufficient number of generations under selection, builds up chromosomes containing more and more of the plus genes, and finally mice result which have one or two pairs of chromosomes containing several or many plus genes. If such mice are then crossed with a race which lacks these genes, the plus and the minus region of the chromosomes of the F_1 received from the self mice would be matched by a region of plus genes in the corresponding chromosomes from the selected line. These regions would segregate as units for the most part, and so the appearance in F_2 of mice with large areas of white on the forehead could be accounted for. This method of accounting for the results of selection and the results of the cross is not put forward as the final explanation, but only to illustrate the kind of modification that must be made in the multiple factor hypothesis if it is to apply to this case.

There are—on modern genetic theory—only two possible ways of looking at the problem presented by the flies and by the mice. Either stable genes exist, so arranged in the foundation animals and, in the case of the mice, in their unselected descendants, that their presence is not manifest externally, or some gene (or genes) has changed during the course of the selection experiments. Existing data on the rate of mutation may well be inadequate, but as they now stand, spontaneous changes in genes occur too infrequently and their effects are too unpredictable to account for the results of the selection experiment. How-

ever, it may be that future work will demonstrate that spontaneous changes occur much more frequently than present information indicates, perhaps with the rapidity required to account for the effectiveness of selection in these cases. Speed alone, however, is not sufficient; direction is also important, for some of the spontaneous changes at least must be in the direction of selection. They must, moreover, continue increasingly in that direction. But before discussing this point to a greater extent, let us consider the hypothesis that the genes for larger areas of white (or larger numbers of bristles) were in the chromosomes of the foundation animals. If this is so, it should be possible to demonstrate this fact in some way other than the changes produced by selection.

The phenomenon of genes present in chromosomes, but hidden from observation, is too familiar to require discussion for its own sake. There are, moreover, several well-known methods by which hidden genes may be brought to light, but which can be applied in this case with great difficulty, if at all. Indeed, if it were not known that genes deliberately hidden in a population can be recovered and if it were not possible to bring genes to light whose existence in a population is not suspected, the fact that selection is able to produce change might be referred to some other mechanism than the sorting of genes. Moreover, the genes have been shown to be such stable entities that, since any selection experiment is limited by practical considerations of space and time to comparatively small numbers of individuals, the factor of gene change is never considered to be of importance. Genes may indeed be lost by accident or intent during the course of a selection experiment, and thus later generations deprived of the complete complement of genes of the foundation stock, but the descendants ordinarily gain no new genes through mutation.

But to say that because selection is able to bring about great change, therefore the genes for the change are hidden in the chromosomes of the reproductive cells of the

animals with which the experiment began, is to beg the question. Nevertheless, if the genes were not there, how are the changes brought about by selection to be explained? Since our present knowledge of mutations indicates that they are too infrequent and too erratic to furnish a suitable explanation of the success of selection, when preformed genes are excluded from consideration, resort must be made to speculation but only to indicate a possible solution of the problem. The substance of the chromosomes is believed to be chemical in nature. Hence, as it is good chemistry to achieve a desired end product by means of intermediate products when the end product can not be achieved directly, and since serial changes are a commonplace of the chemistry of living organisms, it may well happen that selection acts not only by the sorting of preformed genes, as is well established, but also by favoring chemical change in the chromosomes. It may be that a chromosomal condition, d, let us say, varies on the one side to c and on the other to e, but that the amount of variation is small—too small to be detected except by delicate means. The chromosomal condition, d, is therefore stable to this extent. But c may in like manner vary between b and d, while e varies between d and f, and f in turn varies between e and g. The cause of these hypothetical variations is not now important, but if changes of this character occur at definite points in the chromosomes, selection would be effective. Since progressive change occurs only when selection pressure is applied, it may be supposed that one function of synapsis is to prevent spontaneous progressive change by bringing the two chromosomes so close together that neutralization of progress occurs. Selection, on this view, creates no new germinal substance, but it favors its spontaneous occurrence and if in this sense is non-creative, it is creative in the sense that these new substances do not appear without its aid.

The known methods of recovering genes or bringing unknown genes to light are effective when the effect of each gene is readily distinguished from that of its fellows,

or, if this is not true, when linked to genes that are easily recognized or when races that breed true are easily established. But these methods become useless where the effect of individual genes can not be separated one from the other, as is the case with most characters in which the variation is linear and continuous. While in this case the individual genes can not be identified, it is reasonable to suppose that they are present because it can be shown that if they were present, the results of their activity would be nearly, if not quite, identical with the character of observed variations, and with the results of crosses. While it may seem unreasonable to demand more evidence than this, the case of the white area on the foreheads of the mice and that of bristle number on the scutellum of Drosophila present instances that do not conform in many ways to the classical picture. Indeed, practically the only evidence that the genes for 160 odd square millimeters of white on the forehead of the most advanced of the selected mice (or for fifteen bristles on the scutellum of Drosophila) existed in the foundation animals, is the fact that these developments occurred under the pressure of selection. Hence, until the existence of the genes for larger areas of white or of larger numbers of bristles in the foundation animals is demonstrated by some method other than response to selection, the question is not closed. In this situation, those who find it difficult to conceive of any means by which these genes can be hidden in the germ cells of the foundation animals may well be close to the truth in believing in constant small changes in the physical basis of inheritance which can be recognized only as their effects are accumulated by selection. These changes may by some be called "mutations," but if this is done, the term "mutation" has been transferred by successive changes in meaning from the elementary species of DeVries which at one bound developed striking differences, often in several characters at once, to the small fluctuating variations of Darwin in the sense in which Darwin used the word "fluc-

tuating''; *i.e.*, small inherited variations, not in the sense of transitory environmental variations which is in current use in biological circles.[2]

Close contact with the mouse material produces an impression that something of the nature of a creative process is taking place under one's manipulation of selection and matings, not a mere rearrangement of pre-existing genes. The slow gains—barely perceptible in a single generation—point to this conclusion and recall the remark of East and Hayes (1914), who said in a discussion of selection as it related to families substantially constant for leaf number in tobacco, ''Further experiment and larger numbers may show that selection can always cause a change in the mean, but will be necessarily so slight that it can be detected only in a long-continued experiment and enormous numbers.'' This concept of East and Hayes may not be entirely fulfilled by the two experiments with which we are concerned—certainly not as regards numbers—but they demonstrate that a slight shift of the mean in each generation over enough generations becomes at length a change of considerable magnitude. When this fact is considered, together with the extent of variation at the beginning of each experiment—which in the case of the flies was almost negligible in amount—and the behavior in crosses of the stocks developed by selection, the results indicate that continued experimentation along the lines developed by these two experiments may throw much light on evolutionary processes. This conclusion is of the greater importance because most mutations, by reason of their nature, do not supply the kind of change necessary for

[2] Darwin is perfectly clear as to his meaning, even though he thought that response of the non-reproductive parts of the body to the environment might be transferred to the reproductive cells, a view that was adopted only because, in an ignorance of the cause of variations of which he was profoundly aware, this means of producing changes in the productive cells seemed likely. He is exceedingly abrupt in his dismissal of transitory responses to the environment as a basis for selection, saying merely—''Origin of Species,'' page 14—''Any variation which is not inherited is unimportant for us.''

the progressive adaptation of a form to its surroundings or for progressive advance in structure. More bristles or larger spots may not of themselves be of advantage to their possessors, but the amount of change obtained in these characters suggests that the methods used to produce them may be equally successful in nature wherever the opportunity for evolutionary improvement exists. But however this may be, they certainly can not fail to effect improvement in plants and animals useful to man.

If the experiments which have been presented in this paper are accepted as evidence that selection can bring about unlimited change, the barrier raised by the view which assigns limited powers to selection is removed and the way cleared for further progress. The question in which I am primarily interested is the amount of change which may be accomplished by selection, rather than the mechanism by which these results are accomplished. *From this point of view, it is immaterial whether that which is developed by selection was hidden in the germ cells of these individuals with which the experiment began, or whether it is a new creation.* Moreover, if it proves true, even in a few cases, that the amount of change which selection can accomplish is unlimited, the question of why this is so can be left to the future. Every evolutionist is interested in obtaining a full understanding of the powers of selection, because if man is able, not only to form new combinations of genes with resulting benefit to himself but, as Darwin and his earlier followers supposed, is able also to develop valuable characteristics from minute beginnings, it may be possible to gain further insight into the way in which natural selection operates. I believe that we have accepted too easily the view that selection is without power to facilitate the creation of new inheritance. Had the Wrights accepted their predecessors' failures as we have accepted the failures of Johannsen and others, we should not now be encircling the globe with airplanes. Certainly the experiments on flies and mice demonstrate that selection is by no means to be cast into

the discard as a creative agent in evolution. Darwin's view that selection has power to advance the creation of new inheritance finds support in these experiments and may eventually regain its former standing as a factor in organic evolution.

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NOTES ON THE CLEAVAGE RATE OF SCAPHIOPUS BOMBIFRONS COPE, WITH ADDITIONAL REMARKS ON CERTAIN ASPECTS OF ITS LIFE HISTORY[1]

ALBERT H. TROWBRIDGE AND MINNIE STAMBAUGH TROWBRIDGE

Introduction

Despite the fact that the several species of spadefoot toads have been known and studied by naturalists for many years, there are still numerous aspects of their life histories which are but slightly known. This is due, perhaps, to the unpredictableness of the breeding congresses, to the relatively short time the toads remain in these congregations and to the secretive habits of the adults during the greater part of the year. Wright (1932) has reviewed in detail the literature pertaining to the life history of the solitary spadefoot, *Scaphiopus holbrookii,* while Gilmore (1924) and Smith (1934) have considered *S. bombifrons* in more or less detail. None of these workers give any information concerning the minutiae of the early developmental stages, nor can we find any account of them in the literature available to us.

Due to the fact that we have had an unusual opportunity to study *Scaphiopus bombifrons*[2] for the past three years, we are taking this opportunity to present a general

[1] Contribution from the Zoological Laboratory of the University of Oklahoma, n. s. No. 163.

[2] We are using the specific name *bombifrons* with some doubt. Smith (1934) has discussed the validity of this name and infers that *hammondii* ranges only into the western part of the state. Our specimens compare favorably with those taken some miles south of Norman and identified as *bombifrons* by Smith. The mouth parts of our tadpoles, however, are not like those pictured by Gilmore (1924, p. 7, Fig. VI) and by Smith (1934, Plate XII, Fig. 1) for *S. bombifrons.* They are more nearly like those shown for *S. hammondii* by Wright (1929, Plate I, Fig. 3). Which name will be eventually accepted awaits a thorough study of the genus and an evaluation of the larval and adult characters.

picture of the early development and to record additional notes regarding several phases of the natural history of this form. A detailed cytological and embryological analysis of development is being undertaken by one of us (M. S. Trowbridge) and will appear elsewhere at a later date. The writers are mutually responsible for the data presented in this paper.

Materials and Methods

Spadefoot toads are generally distributed throughout Cleveland County, Oklahoma, and other sections of the state, but they are seldom seen, except during the breeding season, when they congregate in great numbers in certain localities. A few scattered specimens may be found in the temporary pools formed in buffalo wallows on the high prairie, but they have been taken in greatest numbers from a large temporary pool formed in a wheat field about one half mile south of the University of Oklahoma campus.

On the night of May 3, 1934, more than 600 specimens were collected and brought into the laboratory. Several clasping pairs were isolated, and from the eggs laid by a single female the initial data on the cleavage rate and early development were obtained. The results secured at that time were verified in 1935 and again in 1936.

We have found it advisable to isolate the clasping pairs, placing a single pair in a culture dish eight to ten inches in diameter containing two to three inches of water. It is necessary to keep these dishes in the dark, although an occasional pair of toads will produce eggs when kept in a lighted room. We have also found that gravid females will be clasped by males when separated individuals are placed in culture dishes. Under laboratory conditions egg-laying has generally started between three and four o'clock in the morning and has been continued until late in the afternoon of that day. Eggs laid during the latter part of the afternoon seldom develop, due perhaps to the failure of the male to produce sperm or to

other conditions, such as oxygen shortage, change in pH, etc. It is true, also, that eggs produced by certain females never develop, but in general approximately 90 per cent. of the isolated pairs have produced fertile eggs.

The eggs from which our cleavage studies were made were removed from the culture dishes immediately after insemination, placed in Syracuse watch glasses, and observed almost continuously for fourteen hours and for several days thereafter with binocular dissecting microscopes. Different stages were preserved at appropriate intervals and from their study, combined with direct observation of the living material, it is possible to tell almost to the minute the time at which different embryonic structures appeared.

Other eggs were placed in finger bowls immediately after insemination and allowed to develop into tadpoles. The temperature range of the water in both watch glasses and finger bowls was between 23 and 27 degrees Centigrade, the approximate range of temperatures in the temporary pools. Due to the rapid rate of development and the necessity for almost constant attention to the eggs developing in the laboratory, we have been unable to check fully our laboratory studies with field observations. However, a partial check was possible in the spring of 1936, and the results indicate that the rate of development of laboratory material is closely approximate to development under natural conditions. This applies particularly to embryos observed from the time of hatching up to and including metamorphosis. It is significant that eggs which developed in the laboratory completed metamorphosis within the same length of time as those which developed under natural conditions in Colorado, as reported by Gilmore (1924).

LIFE HISTORY

Breeding Season

Writers on the subject seem pretty generally agreed that the time of breeding of this species is dependent upon

the rains of late spring or early summer. In fact Smith (1934) writes: "It is probable that they breed any time coincident with the first heavy spring showers after the middle of spring, even though it be so late as the last of summer. It has never been proved that they lay more than once a year." Our evidence, although substantiating this statement, is not in full accord with it. Neither is that presented by Gilmore (1924) when it is critically analyzed. The data gathered by the latter and by us indicate a well-marked breeding season with rather definite seasonal boundaries.

To quote from Gilmore (1924):

> Near Colorado Springs, Colorado, where these observations were made, there is usually a short season of heavy rains early in the summer. This season is preceded and followed by periods of dryness. In 1921 the first appearance of the spadefoot was May 31. On July 5–6 a second rain brought out another group. In 1922 the first appearance was June 1. A very few others appeared later. In 1923, June 15 was the date of first appearance. In 1924, after thirteen days of continuous drizzling rain, the first spadefoot was observed May 31. At nine o'clock in the morning eight male specimens were found. At three in the afternoon twelve were found. Mating had begun. At nine o'clock in the morning of June 1, mating animals were present in hundreds. A few eggs had been layed. June 2, eggs were abundant and some individuals had started back toward the hills. June 3, only a small group remained in the water.

When these data are carefully examined it appears that throughout the four-year period the majority of *Scaphiopus* appeared not earlier than May 31 and not later than June 15, excepting the unusual appearance of July 5–6, 1921. The evidence strongly favors the idea of a definite breeding season.

Our data for the past three seasons are in full accord with those of Gilmore. Following a heavy rain on April 4, 1934, two specimens were taken from a deep ditch west of Norman, but none were found elsewhere. On the night of May 3, 1934, the spadefoots were out in large numbers. In 1935 the spadefoots did not appear until April 28, when they were abundant in the ditches and in the large pool south of the campus. They appeared on the night of May 8, 1936. In this area at least, and for as long as we have

studied it, the spadefoots have had a breeding range not to exceed two weeks. It probably varies in other regions of the state, according to the spring rainy season.

As in Colorado, the time of breeding appears to be directly correlated with the season, and also with the amount of rainfall. Gilmore does not give rainfall data for the years in which his observations were made, but we have compiled these data for this area from the official weather station situated in Norman. They are summarized in Table I.

TABLE I

THE DAILY AMOUNT OF RAINFALL FOR THE MONTHS IMMEDIATELY PRECEDING THE FIRST BREEDING CONGRESSES FOR A THREE-YEAR PERIOD. THE RAINFALL RECORD FOR EACH YEAR IS TABULATED ONLY UP TO THE DATE OF THE BREEDING CONGRESS OF THAT YEAR

Month	1934	1935	1936
April 4	1.55 inches		no
" 15	.23 "		rains
" 17	.35 "		in
			April
" 18	.02 "	1.05 inches	
" 19	.00 "	1.55 "	
" 25	.60 "	.20 "	
" 27	.19 "	.00 "	
" 28	.00 "	1.20 "	
May 2	.00 "		1.07 inches
" 3	2.00 "		.00 "
" 8			2.59 "
Total rainfall ...	**4.94** inches	**4.00** inches	**3.66** inches

An inspection of Table I reveals a number of interesting facts. First, *Scaphiopus bombifrons* did not breed following the first heavy spring rains of any of the three years. Rather breeding was delayed until at least a total precipitation of 3.66 inches for the preceding five or six weeks had fallen. Second, in all cases a heavy rain was required to bring the toads out in numbers. Although the data are not included in the table, rains averaging from .73 to 1.5 inches fell after the breeding dates, some of them occurring as late as the latter part of May for all three years. Spadefoots have never appeared after the first breeding congress, except in 1936, when a gravid female was picked up in a pool formed in the Norman streets following a .23 inch rain, May 23. Neither have they appeared after heavy rains in June or subsequent months.

It seems to be certain that *S. bombifrons* in Oklahoma has a well-marked breeding season, and that this season is definitely correlated with climatic factors and time of year. We are inclined to think late appearances, as the July appearance of Gilmore, and the August appearance reported by Smith (1934), to be exceptions to a rather definite rule. The evidence indicates a hereditary breeding pattern, more or less influenced by climatic conditions at the time the eggs are ready for laying. It remains to be shown how long spadefoot females can retain ripe eggs and have them fertilizable when laid. It is well known that certain other amphibia resorb the eggs and sperm if favorable conditions do not prevail during the breeding season.

Workers who have the opportunity should carry on long-time observations on spadefoot appearances in favorable localities. The evidence which they would obtain might strengthen our position or show that we are in error. It is to be noted that the geographical location may influence the breeding season, for Gilmore found the spadefoots breeding almost a month later in Colorado than we have found them breeding in the vicinity of Norman. This may be a temperature effect which has not as yet been seriously studied. During the three years in which our observations have been made, the minimum and maximum temperatures on the breeding dates have been about 57 to 77 degrees Fahrenheit, respectively.

Breeding Habits

Gilmore (1924) found that in Colorado the males sometimes appear at the breeding pools in the daytime, as early as eight o'clock in the morning. We have recorded the daylight appearance of spadefoots on only one occasion. On the afternoon of May 4, 1934, following a two-inch rain, spadefoots were heard calling about four o'clock in the afternoon. By midnight they were present by the hundreds in the pool south of the campus and mating was well under way. This and other observations incline us

to agree with Goldsmith (1926) that the first arrivals at a pool probably attract by their voices others from some distance away which might not otherwise find suitable breeding pools.

We have made no observations on the part played by the voice of this species in sex recognition. Superficial attention only has been given to this and kindred phases of spadefoot natural history which are in need of further elucidation. It is indicated that the voice may play an important part in bringing males and females of *S. bombifrons* together. Bragg (1936) has reviewed the literature concerned with sex recognition in amphibians and tentatively concludes that voice plays no part in sex recognition in *Bufo cognatus*. We hope to deal further with this question if opportunity permits.

In the vicinity of Norman, spadefoot males always arrive at the breeding pools first and remain until after all the females have disappeared. A quantitative study of the sex ratio in *S. bombifrons* would be of help, perhaps, in explaining this. General observations lead us to think that there are many more males than females at any breeding congress. On the night of April 28, 1935, we found numerous males present in the large pool south of the campus when we visited it at seven o'clock. No females were seen. To quote from our field notes:

> There was a huge congress in the temporary pool where we found them last year. At 11: 15 P. M. there were very few clasping pairs but by midnight the number had increased greatly. By 1: 30 A. M., of April 29, clasping pairs were abundant. The male of this species gives his call while floating outstretched on the water. It would seem as though they wait for a mate, as we have never seen more than one male and one female together—never a free-for-all as in *Bufo*. *Bufo* stays in close to shore, but *Scaphiopus* must lay its eggs in the deeper parts of the pool as clasping pairs are much more numerous in water nearly knee-deep, the maximum depth of the pool.

We have been unable to observe this pool on the day following a congress as it has been necessary to confine ourselves to the developing eggs. Gilmore (1924) found that egg-laying may continue throughout the day, and that the congress may remain intact for as much as three days,

if rain is continued. Ortenburger (1924) found that in Arizona *S. hammondii* left the breeding pools in the daytime and returned at night. We have never found a congress to be in session for more than one night, although it is possible that pairs which do not mate until nearly daylight may produce eggs throughout the day, and perhaps during the early part of the evening. However, we have never taken clasping pairs the second night. In 1935, as mentioned above, the spadefoots appeared early in the evening of April 28, but mating did not take place in numbers until early morning of April 29. We returned to this pool about nine-thirty o'clock in the evening of April 29 and found about fifteen males calling woefully. No females were seen. At one-thirty o'clock on the morning of April 30 we returned to the pool, but were unable to find a single spadefoot. In the vicinity of Norman, at least, the breeding season is of short duration. Storer (1925) found the same to be true in California, as he remarks, "Once concentrated spawning is evidently accomplished with speed as indicated by the large number of eggs in similar stages of development found in the ponds near Santa Maria following the first heavy late spring rain."

In 1936, the spadefoots first appeared on the night of May 8. The large pool, to which mention has been made previously, was not formed, due in part to agricultural improvement and also to lack of sufficient rains previously to thoroughly soak the ground. However, they were generally prevalent in the ditches within a radius of several miles of Norman, but in relatively few numbers as compared to previous years. This is explainable, perhaps, on the assumption that spadefoots do not reach sexual maturity until at least the spring of the second year of life, and that the young of 1934 failed to survive the drouth of that year. Further evidence will be advanced to defend this point in a later section of this paper.

The breeding behavior has been considered by a number of workers, Ortenburger (1924), Gilmore (1924),

Smith (1934) and others. They are generally agreed as to its essential features. Shortly after arrival at the pool mating begins (Gilmore, *op. cit.*). In the Norman area mating apparently does not begin for several hours after the males arrive at the pools. The males lie outstretched and give their call and, we believe, wait for the females to come to them.

Before mating, individual toads are easily captured, but after clasping has taken place the toads become more wary. The greater part of a single swimming individual is easily discernible, but only the heads and eyes of mated pairs show when a light is flashed upon them. These pairs are quick to take alarm and may submerge almost instantly, but reappear on the surface within a minute or two at no great distance from where they disappeared. Amplexation is inguinal, and the eggs must normally be laid while the toads are swimming. We have found egg masses attached to submerged sticks, clumps of grass, etc., but never on the bottom of the pools. Our observations on the size and shape of the egg masses are not of any great value as we were unable to make them before the larvae were at the point of hatching. Gilmore (1924) found that in Colorado "the egg masses vary in size. Large masses contain 200 to 250 eggs, smaller ones 10 to 50. The mass is attached to submerged vegetation or to any object protruding from the bottom. The mass is elliptical in shape."

DEVELOPMENT

Early Cleavage

Although the literature of natural history contains many references to the rapidity with which the spadefoots develop, there appears to be no published work on the embryology of these forms. This is indeed surprising when one considers the extensive use of amphibian material in both general and experimental embryology. In Europe many biologists, as the Hertwigs, Brachet and Roux, and in America, especially Thomas Hunt Morgan,

have contributed largely to a clear understanding of the normal developmental processes in the common European frog *Rana temporaria* and the American grass frog *Rana pipiens*. In general, the developmental history of each of these forms is about the same.

The first cleavage begins from two and one half to three hours after insemination, and the succeeding cleavages appear about one hour apart, gastrulation being completed in about 36 hours. Within a week or two after insemination the young larva (6 to 7 mm long) leaves the egg and remains for a week or ten days resting on its side on the mud or attached to some object by the mucous gland. During this period the functional larval mouth develops, the eyes appear, the gills enlarge, general increase in size takes place, and important internal changes occur. Nourishment is derived from the yolk mass. Metamorphosis takes place in from two to three months after insemination, depending on the food supply and temperature. In contrast, in the bull frog, metamorphosis does not occur until two or three years after hatching, depending on the locality.

The story is much different for the spadefoot toad. The first cleavage of the egg begins about 30 minutes after insemination and is completed in nine minutes. A one minute interval occurs before the second cleavage begins. It is completed in about eight minutes, and a minute and a half later the third cleavage furrows appear and are completed in about nine minutes. The egg then rests for almost ten minutes. The fourth cleavage is completed in four and one half minutes, after which the egg rests for nearly 11 minutes. The fifth cleavage is completed in three and one half minutes. Following this, there is a rest period of about 11 minutes before the sixth cleavage furrows become visible. From this time on, the cleavages are so irregular and succeed one another so rapidly that it is impossible to follow them.

It is to be noted that as the time consumed for cleavage decreased, the time spent in rest increased; at least, this

conclusion holds true for the first five cleavages. The significance of this is not evident.

We have not compiled figures on the regularity or irregularity of the cleavage planes at this time. In general, however, the first two cleavages are equal, meridional and at right angles to each other. The third cleavage is equatorial and divides the egg into four smaller blastomeres at the animal pole, and four slightly larger blastomeres at the vegetal pole. Succeeding cleavages steadily reduce the size of the blastomeres, although as in most amphibian eggs the cells at the animal pole divide more rapidly than those at the vegetal pole. For material to demonstrate amphibian embryology to either freshmen or advanced students, nothing equals the eggs of *Scaphiopus*. The light brown animal pole, and the creamy white vegetal pole, with the deep, definite cleavage lines and the almost geometrical regularity of the blastomere arrangement up to the 32-cell stage make this a form which can not be too highly recommended for class use. To secure material, however, it is necessary to give one's undivided attention to the job.

We have taken the egg from insemination to the beginning of the sixth cleavage. The entire process has consumed approximately one and one half hours. In *Rana pipiens,* the same stage is not reached until nearly eight hours after insemination. The comparative developmental ratio, then, for *R. pipiens* and *S. bombifrons* is approximately as one is to six (1:6).

Gastrulation and Later Development

There is a slight change in this ratio up to the end of gastrulation. At this time the embryo is still almost spherical in form, but has increased somewhat in size and the medullary plate and neural ridges have appeared. Due to the rotation which has taken place, these structures now occupy their definitive dorsal position and the blastopore is posterior. The latter has become quite small, but is still circular and the yolk plug is faintly

visible. This process is completed in about 36 hours in *Rana*. It is completed in eight hours in *Scaphiopus*. For this period, the developmental ratio is as one is to four (1:4).

Following the completion of gastrulation, the comparative developmental ratios change markedly. The embryo of *Rana* develops comparatively slowly and under the most favorable conditions does not hatch for at least a week. The development of *Scaphiopus*, on the other hand, is so rapid as almost to be called cataclysmic. The neural folds are strongly developed in nine hours and practically closed by eleven hours after insemination. At the same time the sucking disc, branchial arches and optic rudiments become evident. Forty minutes later the neural folds are completely closed, the optic vesicle is well defined, the olfactory pit is present, the tail rudiment is well marked, the outer egg membrane is completely sloughed off, and the embryo is rotating within the inner membrane. By twelve and one half hours the first muscular movements (spasmodic twitching of the embryo) occur, and within thirty hours after insemination the embryo escapes from the inner membrane. *Rana* requires from seven to twenty days to complete the same process. The comparative developmental ratio for this period, then, is as one is to six to eleven (1:6–11).

After hatching the spadefoot larvae rest on the bottom for from eighteen to twenty-two hours. During this period the eyes develop and there is a general elongation of the body. At the end of this period the larvae are able to orient themselves and swim actively about and feed on bits of algae. *Rana* requires a week or ten days after hatching to reach this stage. The ratio is as one is to eight and five tenths (1:8.5).

Further development has been ably described by Gilmore (1924) as follows:

> The newly hatched tadpole is a trifle less than one fourth of an inch in length. Within less than five days it has doubled in size. Within another five days it has obtained a length of one inch. The legs then begin to de-

velop. Fifteen days later it has reached its maximum size. Two and one half inches is the maximum length of the majority of adults in any tadpole community. A small minority attains three to three and three quarters inches in length. At about the thirtieth day after egg-laying, arms begin to appear, and the process of transformation begins to be evident in all parts of the animal. The complete absorption of the tail and the completion of remodeling all structures into adult form is accomplished by the fortieth day.

We have observed that change in body and in external characteristics appear somewhat earlier than as reported by Gilmore. The tadpoles become lighter in color by the fifteenth day and by the end of the twenty-fourth day are assuming some of the adult characters. The skin becomes still lighter, rugose and mottled, and the body shape is noticeably changed, as are the mouth parts. Shortly after the arms appear full adult coloration is attained, the eye is of the adult type and the mouth parts are rapidly transformed to the adult condition.

As noted by Gilmore, all tadpoles known to be of the same age are not of the same size. "From ten to twenty per cent. of specimens known to have hatched at the same time are considerably larger than average. Some may be two or three times the size of the average. These larger forms are frequently found with smaller ones half eaten." It is quite obvious that those tadpoles which grow the fastest have the best chance of survival, due in great part to the food habits of the older tadpoles.

Food Habits of the Tadpoles

Gilmore (1924) was unable to determine the food habits of the smaller tadpoles, but believed them to be strictly carnivorous in nature. Our observations for the three-year period indicate that the reverse of this is true. Within forty to fifty hours after the eggs are laid the young tadpoles are eating algae and require an immense amount of it per individual. Apparently they are restricted to a vegetable diet for a period of two to four days, as we have never found them eating dead tadpoles until they were at least six days old. From this time on, however, spadefoot tadpoles must have meat to develop

normally, but in addition, must be supplied with algae. Throughout their entire growth period the tadpoles are extremely active feeders. There is a constant stream of food going in on the one end, and an almost constant discharge of feces at the other.

We have fed raw beef-liver, lean beef, hard-boiled egg yolk, fragmented grasshoppers and lettuce in addition to the algae. In 1935 we transferred the tadpoles to a large tank at the end of the fifteenth day of development and kept them solely on a meat diet. Development was not at all normal. The tadpoles increased rapidly in size and some attained a length of four to four and one half inches. The legs did not appear at all in some of them, and broke through a week or so later than normal in others. Generally the legs emerged enclosed in a membranous fluid-filled sac. We interpret this as a response to a dietary deficiency. Few tadpoles of this crop underwent metamorphosis. Rather, between the thirty-eighth and fortieth day of development the majority of them died. Death was preceded by a general increased turgidity of the body of the tadpoles, evidently due to the pressure of absorbed water. The evidence seems to indicate that although the spadefoot tadpoles are carnivorous in their food habits for the most part, they must have algae or other green vegetable material to undergo normal development.

There can be no doubt that the spadefoot tadpoles are cannibalistic as they increase in size. In 1934 we had a large number of tadpoles in a tank and noticed that the number appreciably decreased from day to day. The decrease in numbers was greater than could be expected from the number of tadpoles which died from other causes. During this spring also we placed about 200 spadefoot tadpoles in a tank which contained approximately 1,000 *Bufo cognatus* tadpoles. Before many days none of the smaller *Bufo* tadpoles remained. Although we have never observed the spadefoot tadpoles make a kill, there is no question but that they do attack and eat smaller living

tadpoles, both of their own and of other species. In nature it has been found that spadefoot tadpoles also eat "smaller tadpoles, shrimps, and insect larvae" (Gilmore, 1924). This author made some interesting observations on the length of the intestine of spadefoot tadpoles which are presented verbatim:

> The tadpoles of most frogs and toads are herbivorous and therefore have very long intestines. As they transform to the adult condition they take only animal food, and the long intestine is replaced by a short one. In the spadefoot the change from a long intestine seems to take place before the beginning of transformation. In fact, some specimens seem never to have had a long intestine. It seems probable that the spadefoot tadpole is departing from the traditions of its ancestors and relatives and adjusting itself to a new type of diet. This adjustment is approaching perfection in the jaws, lips, roof of the mouth and jaw muscles. The long intestine character has not been eliminated, but is in process of elimination. It seems to persist during early tadpole life and is later supplanted by a short intestine. The short intestine character will be subject to a wide range of variability until it has firmly established itself on the race.

Later Development and Habits

Transformation takes place when the developing toads have attained a body length of sixteen to nineteen millimeters, and when the tail is about fifteen to twenty millimeters long. Complete absorption of the tail requires only three to four days. At the beginning of the transformation period the arms are well developed and the body is shaped essentially like that of the adult. At this time it is necessary to put enough sand in a part of the culture dishes or tanks so that the transforming tadpoles may leave the water. Failure to do this causes a delay in metamorphosis which may be prolonged for more than a week.

Shortly after leaving the water, the young toads are ready to eat, even though they may have tails nearly as long as the body. The burrowing reaction is also developed, although the small toads rarely burrow before the tail is less than one half the body length. However, two individuals are definitely known to have burrowed when the tails were fully as long as the body. Cope (1889) found transforming tadpoles in a lake in Idaho. He writes that

they were "engaged in eating grasshoppers and I detected several specimens with the entire insects in their mouths. In some instances the grasshoppers' bodies were too large and projected from their mouths. These precocious larvae were evidently air-breathers, and hopped about, presenting a curious appearance as they dragged their large tails after them."

On June 14, 1936, at 10:30 P. M., we placed a number of recently transformed toads with tails between a third and a half the length of the body, in a large box, the bottom of which had been covered to a depth of three or four inches with moist sand. By 11:45 P. M. all had burrowed out of sight.

In burrowing their habit is to sink backward into the sand, digging from the rear by means of the "spades" on the hind feet, sidling back and forth and shuffling the hind limbs in quick and erratic movements which enable the creature to conceal itself below the surface of the ground in but a few moments (Smith, 1934).

At 1:30 A. M., June 15, several of the small toads mentioned above had come out of their burrows and were hopping about the box. Others were sitting in a shallow pan of water. When a number of small ants were released in the box, the toads hopped after them and ate them with seeming relish. The first attempts to catch moving insects, almost without exception, met with failure. This was due to inability to judge distance, and coordinate the body movements and tongue movement with the moving insects. However, their proficiency increased with practice.

During the first few days our toads were kept in the box, they were fed small, slow-moving insects. Termites were eaten avidly, as were small, newly hatched isopods, and small species of ants. By June 17, all toads were finished hunters, although several of them were more aggressive and more adept at catching insects. By this date, also, their eyesight had developed remarkably and the slightest movement of a small insect some six to ten inches away would cause the toads to give chase. Approximately

90 per cent. of all attempts to capture insects are successful. When a miss is scored and the tongue becomes covered with sand the hands are used to remove the debris. They are also used to orient large insects before they are swallowed.

As previously pointed out, the tadpoles develop at varying rates, and at any one time a certain percentage will be larger than their fellows. Despite this, however, metamorphosis takes place when the toads are nearly the same size. Following transformation, individual toads increase in size at varying rates. As this is written (September 30, 1936), some of the toads which metamorphosed on June 9 have doubled in size, *i.e.,* are from 24 to 28 millimeters in body length, but an equal number have gained only a third in length. As adults average slightly larger than 50 millimeters in body length, it would appear as though our toads were between one third and one half grown. Of course in nature the toads might or might not grow as rapidly as they have in the laboratory, depending primarily, we presume, on the food supply. If this be true, one would not expect individuals of *S. bombifrons* to obtain sexual maturity before the end of the second year.

About June 20, 1936, the toads had increased about one third in size, on the average, and we ceased to hand-feed them. Rather, the box containing the toads was placed under an electric light at night, and the toads were allowed to shift for themselves.

Apparently any moving insect will be seized, and nearly all of them will be eaten. However, certain small species of carabid beetles, leafhoppers, red harvester ants and a scattering of other undetermined forms will no longer be attacked by the toads. The learning process is unquestionably one of experience—an unpleasant one, if we can judge by the reactions of the animals. When an unpalatable insect is taken, the toads hop and roll around frantically and paw at their mouths with their hands in an effort to dislodge the insect. Only two or three experiences of

this nature seem to be necessary before that particular species of insect will no longer be attacked.

We have definite records of the toads eating representatives of eight identified orders of insects, in addition to certain other arthropod forms. They are as follows:

Diptera (house-flies, mosquitoes, midges, etc.)
Coleoptera (chrysomelids, coccinelids, scarabids, carabids, staphalinids, and others)
Hymenoptera (small ants of several species)
Homoptera (many species of leafhoppers)
Neuroptera (lace-wings)
Orthoptera (small grasshopper nymphs, small mantids, etc.)
Lepidoptera (miscellaneous small moths)
Isoptera (termites)
Spiders of several species
Small isopods

What the food of the growing toads may be in nature is not known. We presume that the greater bulk of it would be of insect forms which are most numerous on the prairie and about the cultivated fields. This would include most of the groups we have listed, and in addition we should expect to find more species of the Hymenoptera and many Hemiptera, forms which are common and numerous in the prairie regions. More work needs to be done on this important activity in the life of spadefoot toads.

As to the habits of the captive toads: They leave their burrows shortly after total darkness has fallen, although rarely an individual appears before that time. When the light is turned on they hop about excitedly for a few moments, but soon settle down to the serious business of feeding. Competition is keen and the larger individuals will often jump at the smaller ones and attempt to wrest from them recently captured small insects. Sometimes they are successful, but more often not. In so far as our toads are concerned, the statement by Smith (1934) that the toads are "strongly negatively heliotrophic and will frequently begin to burrow as soon as a light is thrown on them (when not breeding)," does not apply. Instead, the toads seem to know that with the coming of the light,

the time of feeding is at hand. We have not observed adults in nature at any time except the breeding season, but believe that they would react as indicated by Smith.

The small toads may feed throughout the night, or only for a few minutes, depending on the abundance of insects. If insects are numerous, the toads eat their fill within a few minutes and then burrow into the ground. It is interesting to note that they may occasionally return to the burrow where the previous day was spent, but most often they burrow in at any convenient place. They burrow so rapidly that they may sometimes be totally hidden within three to five minutes.

The intense heat and low humidity of the present summer (1936) have caused the surface of the sand to dry out during the daytime, and thus the toads are imprisoned. We have left them in this condition for as much as four days without any apparent ill effects. When water is poured on the dried soil protesting squeaks are heard and the location of the burrows indicated by the air bubbles which arise from them. The same squeaks may be heard when pressure is applied to the sand immediately over a burrow. At all other times the toads are silent.

The drying of the surface layer of soil leads us to believe that a drought year may deal harshly with the spadefoots. Fewer individuals may complete metamorphosis, and small, recently transformed toads may be trapped in their burrows and starved to death. In addition, if the spadefoots do not breed until the second year of life, losses occasioned by drought conditions should be reflected in the numbers of individuals in the breeding congresses two years hence. The summer of 1934 was a drought year, and in the spring of 1936 very few spadefoots were found in the breeding congresses. If our reasoning is right, we might expect to find few toads in the breeding congresses of 1938.

If the habits of the adults were better known and if one could tell where to find them during the year further evidence might be obtained as to the effects of a drought on

the species. Spadefoots are generally believed to appear only after heavy rains and to be more or less confined to their burrows throughout the remainder of the year. Smith (1934) believes that:

> It is probable that after breeding they scatter widely, and since they do not sing, when they do emerge merely for food, it is seldom that they are found. How frequently they do come out at night is not known, but the writer has observed adults at night in the sand dunes near Medora, Kansas, hopping about in considerable numbers in light showers. The evening following this particular one was clear and only a single specimen, an adult, was secured.

Kellogg (1932) has found the young emerging at night during the summer, entirely independent of rain:

> In suitable sandy areas, this nocturnal spadefoot comes out of its burrow during the summer months after it gets too dark for one to see objects without the aid of a flashlight. Along the Powder River near Powderville in Montana, on June 15, 1916, while lying upon my cot, I heard a curious rustling in the dry leaves about our tent. Upon investigation with a flashlight many small spadefoot toads were found. They were hopping about in the dry leaves which were scattered about the sandy soil. When hunted with a flashlight they endeavored to burrow out of sight and but a few minutes were required for them to entirely conceal themselves. These spadefoots make circular holes in the ground and yet in sandy soil it is very difficult to find the place where they have buried down, for in most cases it seems as if they pulled the hole in after them. After the breeding season is over, they take more pains in constructing their burrows, as they are well rounded and resemble somewhat an earthen jar with a narrow top. Around this opening there is present some sticky matter which may aid in the ensnaring of insects. I have usually found this toad most plentiful in sandy areas, especially along the banks of streams though they occur on the elevated plains from Kansas to Montana.

There still remain several phases of the life history of *S. bombifrons* which are dependent on further field and laboratory studies for their solution. Further studies are needed on the natural history of the adults, including their mode of life and their food habits. Laboratory researches might lead to a knowledge of why the tadpoles grow so rapidly and if there is a hormone as yet unknown which influences the rate of growth. Nutritional and physiological investigations as to the factors influencing the death of tadpoles fed only on meat should lead to interesting results. It may be that *Scaphiopus* has a value in experi-

mental zoology which to this time has escaped the attention of biologists as a group.

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EXPERIMENTAL SEXUAL PHOTOPERIODICITY IN THE MALE TURTLE, *PSEUDEMYS ELEGANS* (WIED)[1]

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INVESTIGATIONS in recent years have shown that in many monoestrous, warm-blooded vertebrates, phases of the sexual cycles are conditioned, to a greater or less extent, by seasonal changes in the length of daylight. In the laboratory, these sexual cycles can be directed by increased or decreased artificial illumination (*cf.* Bissonnette (1936) and Marshall (1936) for fairly complete summaries of present knowledge).

The response of the sexual cycles of cold-blooded vertebrates to changes in the daily amount of illumination received is a relatively unexplored field. Among the reptiles, Clausen and Poris (1936) find with *Anolis carolinensis* that sexual activation is accelerated by increased illumination. The experiment to be described indicates that even under apparently adverse environmental conditions, and with the testes in an apparently unfavorable spermatogenetic stage, an increase in the daily amount of illumination will induce in the testes of *Pseudemys elegans* a new spermatogenetic cycle.

I wish to express my gratitude to Professor T. H. Bissonnette for his generosity and to Mr. E. E. Bailey for his many kindnesses.

MATERIALS AND METHODS

Seventeen freshly collected, adult male turtles, *Pseudemys elegans,* were used in the experiment, which was started on November 18, 1936. In addition to normal daylight, eight turtles received artificial illumination from 50-watt bulbs. Artificial lighting was begun each evening

[1] Aided by grants from the Committee for Research in the Problems of Sex, National Research Council, 1936–37, administered by T. H. Bissonnette.

at 7 P.M., and was gradually increased from three additional hours per night to seven additional hours. These turtles were kept in a museum room where the temperature varied between 24 and 10 degrees Centigrade.

Two sets of controls were employed. One set of six turtles was kept in the same room and under the same conditions as the group receiving additional light. This set, however, got no increased illumination; although, it may be mentioned, for the last month of the experiment day-lengths were slowly increasing. Another set of three turtles was confined to a barrel in a dimly lighted part of a cellar room. The temperature of this room was slightly lower than that of the museum (20–5 degrees Centigrade).

One animal from each group was killed on January 7. All the remaining turtles, except one from the group receiving added lighting and one from the group on normal daylight, were killed on January 23. The two remaining turtles were sacrificed on February 5. The testes were fixed in Bouin's and Helly's fluids.

The environmental and psychological conditions under which the turtles were kept would appear unfavorable to the well-being of the animal. Although *Pseudemys* is an aquatic turtle, the reptiles were furnished only shallow pans of water which covered no more than one half an animal. Moreover, a number of females[2] were kept with the males, so that each pen contained from four to ten turtles, while the pans of water accommodated only two animals at a time. For the first month, all the turtles attempted to pile into the pans at the same time, but later the animals ceased to exhibit this behavior. In addition, the groups kept in darkness received no water at all.

During the day, students were passing to and fro before the cages. The approach of any one alarmed the reptiles, and often sent them dashing blindly into the sides of the cages or into each other. Hence, there existed during the day frequent periods of fright.

[2] The number of females on hand was too small to be adequately studied.

The turtles refused to eat any of the food proffered them (vegetables, fruits, fish, liver). After the first three weeks, attempts at feeding were discontinued. Thus, the animals were forced to live on their own food reserves for the experimental period.

As is common with turtles, these were heavily parasitized. The walls of the intestines were almost covered with nematodes, and an intestinal protozoan fauna was abundant.

Results

Animals killed on November 18, at the beginning of the experiment, showed that the spermatogenetic cycle was well advanced. The epididymides were gorged with spermatozoa. In the testes, spermatids sufficient to form a large number of spermatozoa still existed. It should be noted that Bissonnette has shown with birds and mammals that the gonads at the end of their gametogenetic cycle tend to be refractory to stimulation by moderate increases in light. By inference, it is not to be expected that increased light would be immediately effective upon the turtles.

Owing to the nature of the results, it will be more convenient to describe the experimental animals first, rather than to follow the customary procedure. In the turtle killed on January 7, which received additional light, there was found the first signs that a new spermatogenetic cycle had started. The walls of the seminiferous tubules had thickened, thereby reducing the lumina of the tubules.

A slight proliferation of secondary spermatogonia had commenced. By January 23, when six animals were killed, there had been an abundant proliferation of secondary spermatogonia and a transformation of some of these cells into spermatocytes. Mitotic figures of these germ cell types were much in evidence. The spermatozoa of the previous (original) cycle had completely disappeared from the lumina of tubules. Moreover, there was a considerable uniformity in the testicular condition of all

the animals that received added illumination. The difference between the various animals was that, in two animals proliferation had not progressed as rapidly as in the rest. In short, there was in these animals an involution of the original spermatogenetic cycle, and in its place a new cycle had started. In the single turtle killed on February 5, spermiogenesis was completed, and spermatozoa were found free in the tubules. By this date, it was apparent that the food reserves were insufficient to support the sexual cycle, for the testes showed obvious degenerative changes, and the spermiogenesis accomplished was relatively slight. But, nevertheless, completed spermiogenesis was produced by experimental treatment.

The status of the controls was different from that found in the animals receiving additional light. In the turtles kept in darkness, there was no suppression of the original spermatogenetic cycle. Both on January 7 and 23, the tubules showed abundant spermiogenetic transformations, and the lumina of the tubules were filled with spermatozoa. In fact, the histological evidence would seem to indicate that the cycle had been carried beyond its usual limits. Tubules were found so distended with sperm that they approached a diameter of 2 mm (measured from preserved material). Hard lumps caused by these distended tubules could be felt in the fresh testes. However, caution must be exercised in claiming an exuberance of spermatogenesis. It may be that the mechanism for draining the tubules of sperm had been impaired, and the distentions were due to a damming of the spermatozoa within the tubules. Furthermore, while I have examined the mature testes of several species of turtles, and have found no comparable distentions, it may be that these distentions fall within the range of the normal variation of turtles in nature. The condition of the testes of these turtles was also uniform.

The controls receiving only normal daylight were not uniform. The testes of two animals on January 23 were in the same condition as those of turtles kept in darkness,

i.e., there was no inhibition of the original spermatogenetic cycle. In the other two, however, by January 23, the original cycle had been checked. These testes were somewhat less advanced than those found in the experimental animal described for January 7. That is, the walls of the tubules had thickened, and cytolysis of sperm was taking place. But here, no proliferation of secondary spermatogonia had as yet occurred. In the single animal killed on February 5, the testis was markedly disorganized, and no evidence of further spermatogenetic advance was found.

Discussion

While it is known that in certain animals the sexual cycle is not modified by changes in the amount of light received, it is becoming increasingly clear that the cycles of a large number of non-tropical, monoestrous vertebrates can be manipulated by changes in the amount of illumination. It is obvious that light or any other external environmental stimulus can only effect changes in the velocity of the cycle or exaggerate phases of this cycle within an inherent morphological and physiological framework.

In the experiment just described, turtles which received increased illumination (3 to 7 hours) were induced to begin a new spermatogenetic cycle, which had advanced, during the experimental period (November 18 to January 23), to the spermatocyte stage, and by February 5 to a complete spermatogenesis. One cycle was inhibited, and another started. Some controls which received only normal daylight also showed an inhibition of their original cycle; but this inhibition was effected more slowly than in the experimental animals. Moreover, in these controls, spermatogonial divisions had not occurred before the termination of the experiment. Since other environmental factors, with the exception of light, were the same, the difference in the testicular state of the experimental animals and the animals on normal daylight can be clearly attributed to the added illumination.

A priori, one would assume that temperature must play an important rôle in the sexual economy of cold-blooded animals. However, it does not follow, unless proven, that increased metabolism, produced by higher temperature, reflects itself directly upon gametogenesis as an inductor of this process. For instance, I have found in the urodele, *Pseudotrition ruber*, that the production of secondary spermatogonia and their subsequent transformations into spermatocytes and spermatids take place, in part, throughout the winter. Mellish (1936) reported with the horned toad that a temperature of 35 degrees Centigrade (furnished by continuous electric light) had only a very slight stimulating effect, *i.e.,* canalization of the seminiferous tubules, as compared to a temperature of 5 degrees. These arguments are advanced, not to minimize the effects of temperature where they have been demonstrated, but to point out that there is no logical necessity to assume that sexual stimulation is universally induced in cold-blooded vertebrates by an increase in temperature.

With the data on hand, it can not be definitely concluded whether the difference between some of the controls on normal daylight and the controls kept in darkness is due to light or to temperature. The average temperature of the room where the animals confined in darkness were kept was slightly lower than that of the museum room. However, since some of the controls on normal daylight were in the same condition (the original spermatogenetic cycle) as those kept in darkness, and since it was shown that the original cycle could be markedly inhibited by increased light, it would appear that the difference in the amount of light received by the animals in darkness and the animals on normal daylight is chiefly responsible for the difference found in the testes of the two sets of controls. It is planned to carry out further experiments to evaluate more accurately the relative importance of light and temperature. The present experiment demonstrates that changes in the amount of light

received by turtles at the period of the year and under the conditions of this experiment modify the spermatogenetic cycle.

Summary and Conclusions

(1) Increased illumination (3 to 7 hours) started on November 18, 1936, on male turtles at the crest of a spermatogenetic cycle, caused an inhibition of this cycle, and induced a *new* cycle.

(2) Controls kept in a cool room and in darkness showed no inhibition of the original cycle.

(3) Controls receiving only normal daylight, and kept under the same conditions as those receiving additional artificial light fall into two groups: (a) Those with no inhibition of the original cycle; (b) those that showed the beginning of a new cycle, which, however, lagged far behind that of the turtles which got increased light.

(4) The difference between the experimental turtles and the animals receiving only normal daylight is attributed to the difference in the amount of illumination. It is suggested that the difference between the two sets of controls is chiefly due to the difference in the amount of light received.

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SOIL AMOEBA (NAEGLERIA GRUBERI) COALESCE TO FORM A PLASMODIUM

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PENNSYLVANIA DEPARTMENT OF AGRICULTURE

INTRODUCTION

THE purpose of this paper is to show that the soil amoeba, *Naegleria gruberi,* is not an amoeba proper but a stage in the life cycle of a mycetozoan. The author has studied *Naegleria gruberi* for fifteen years in connection with mosaic diseases of plants. In 1920, he isolated some small amoeboid forms from the intestines of aphids feeding on plants affected with mosaic disease. At the same time some leaves of tobacco plants affected with mosaic were washed in bichloride of mercury solution, 1–1000, and placed in sterile Knop's solution for two weeks. The same amoeboid form found in the aphids appeared in the solution, while none were found on healthy leaves treated in the same way. This led the writer to believe that these small amoebae might be the causative agent of tobacco mosaic. Numerous experiments and inoculations were carried out, but the disease seldom appeared in the experiments, so no conclusive results were obtained.

During a morphological study of *Naegleria gruberi,* a cigar-shaped parasite was found in a few of the amoebae. This parasite had a flagellate movement and was about a micron in length. It had the power to round up and encyst. Although the author has never been able to get this parasite to live free of the amoeba, he has often seen small round objects in the nucleus of infected plants. These objects have the same dimensions as the cyst found in the amoeba. The above evidence and the work of Miss S. H. Eckerson (1926) lead the writer to believe that soil amoeba, *Naegleria gruberi,* may be one of the alternate hosts for the mosaic-producing organism.

Methods

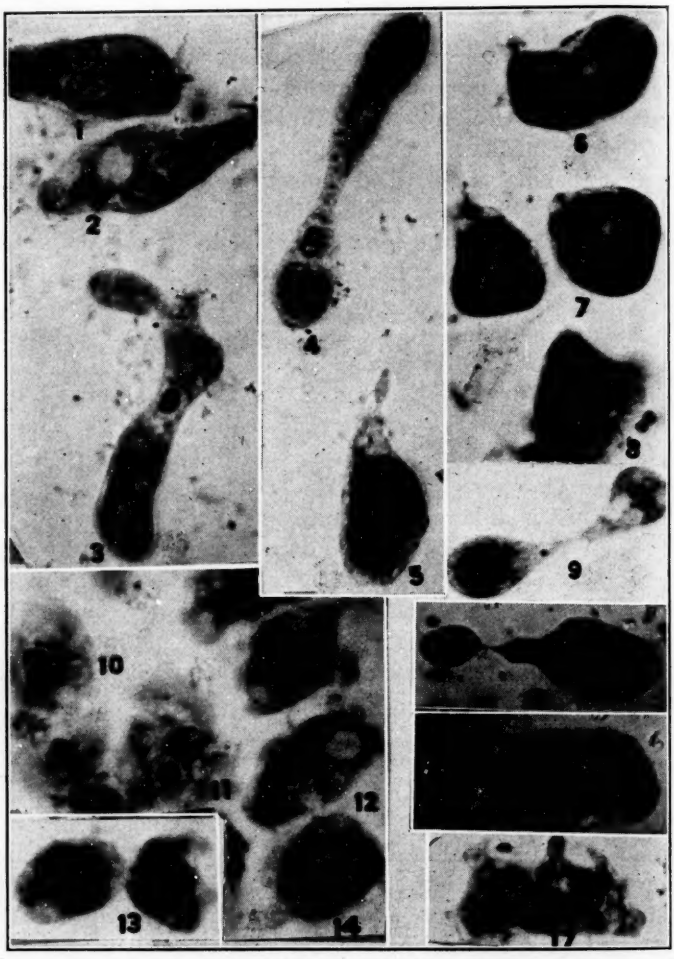
The cultures were made in Erlenmeyer flasks with necks large enough to permit the passage of cover glasses. The latter were used in all examinations to detect the presence of the organism. At first Knop's solution was used for a medium but later distilled water was found to be equally efficacious. The flasks containing the medium and cover glasses were sterilized in an autoclave at 15 pounds pressure for 45 minutes. Leaves or stems of tobacco plants affected with mosaic diseases were cut into small pieces, washed in 1–1000 mercuric bichloride solution in distilled water and allowed to incubate for 7 to 15 days. By repeated transfers, the organism has been maintained in culture for 15 years. To study the soil amoeba, the cover glasses were removed with a flamed bacterial culture needle. They were then wiped on the under side, mounted face up on a slide and examined under the microscope.

For cytological studies, the organism was killed by exposure to osmic acid fumes for one fourth of a minute and then placed in Schaudinn solution (95 per cent. alcohol saturated with mercuric chloride). The fixed material was stained in Heidenhain's iron haematoxylin (short method) and mounted in balsam.

Amoeboid Stage

Morphology of the amoeba: A cyst gives rise to a limax type of amoeba (Fig. 1, 2, 3, 4). The nucleus of the amoeba is spherical in shape with a distinct nuclear membrane (Fig. 1). Between the nuclear membrane and the small karyosome lies a clear area. The karyosome is centrally located and appears as a large compact sphere which sometimes stains homogenously; in other cases the central portion stains much lighter than the periphery. A small deeply stained granule, which may be a centriole, can be seen in the center.

Binary fission: The amoeba may or may not round up just preceding division. The beginning of nuclear division is characterized by an increase in nuclear size (Fig.



Photographs 1–17 inclusive × 2000

PLATE I

FIG. 1. Prophase.
" 2. Late prophase showing two polar caps, granular chromatic polar masses and centrodesmose.
" 3. Prophase with karyosome becoming dumb-bell shape.
" 4. Prophase.

2). During division all movement of the organism ceases (Figs. 10–17). The division of the nucleus is promitotic, that is, large chromatic polar masses are formed within the nuclear membrane and fibrils appear between them. The nuclear membrane becomes more distinct and the peripheral chromatin more evident than in a resting nucleus. The central karyosome then increases in size, elongates and assumes a bent dumb-bell shape. The peripheral chromatin at the same time migrates in the membrane towards the karyosome. Spindle fibers appear between the chromatic polar masses. The eight subequal chromosomes are formed in a regular equatorial plate and later one divides by constriction. The chromosomes then move apart (Fig. 10). The nuclear membrane constricts in the middle and the two daughter nuclei separate. The resting nucleus is reconstructed. At the same time that reconstruction and separation are taking place in the nucleus, division of the cytoplasm starts.

Flagellate Stage

Morphology: The flagellates which arise from an amoeba or cyst spores are pyriform in shape and appear smaller than the amoeba. The nucleus of the flagellate is like that of the amoeba, with a well-defined membrane and central karyosome, and divides promitotically (Fig.

" 5. Endogenous bud containing chromidia almost separated from parent individual.
" 6. Late anaphase.
" 7. Late anaphase.
" 8. Prophase.
" 9. Telophase.
" 10. Late prophase showing two polar caps, granular chromatic polar masses and centrodesmose.
" 11. Late telophase.
" 12. Prophase.
" 13. Nucleus has divided and cytoplasm ready to pull apart.
" 14. Late prophase showing two polar caps, granular chromatic polar masses and centrodesmose.
" 15. Endogenous bud containing chromidia almost separated from parent individual.
" 16. Spindle of late prophase.
" 17. Late telophase daughter nucleus almost separate.

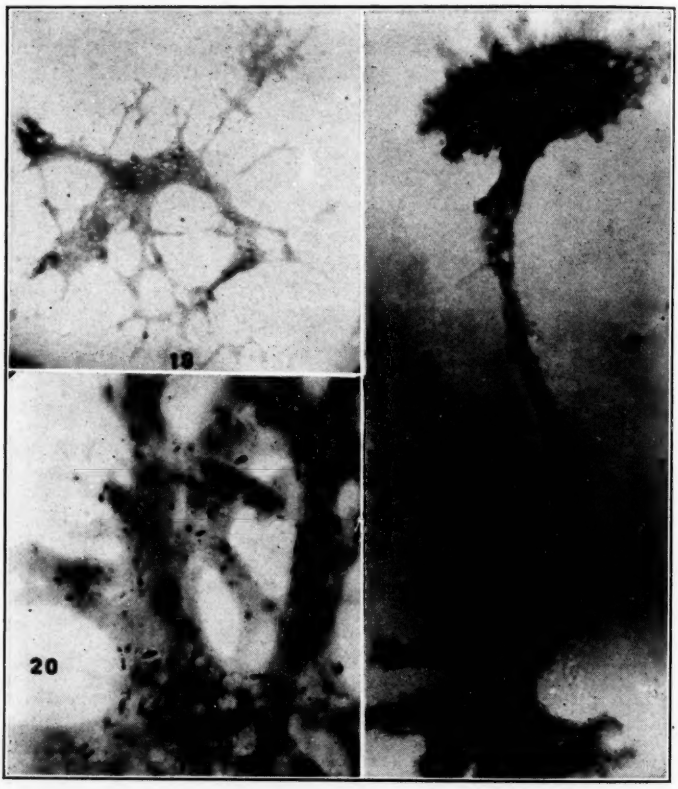


Fig. 18. ×350
" *19.* ×950
" *20.* ×1200

PLATE II

FIG. 18. Fusion of amoebae to form a plasmodium.
" 19. Plasmodium with some of the nuclei in the early prophase.
" 20. Plasmodium with nuclei in all stages of division.

39). The position of the nucleus in the cytoplasm is variable, generally being near the center of the organism, but some forms were found in which it had migrated into the anterior end. In all such cases the organisms were feeding rapidly, and the flagellum had become longer (Fig.

37). The younger flagellates differ somewhat from the older in size and shape, being larger on the average and having a width of one third the length of the body as compared with two thirds in the older organisms. Differentiation into ectoplasm and endoplasm can be seen only when the posterior end of the flagellate becomes amoeboid (Fig. 36). The endoplasm is filled with food vacuoles which contain bacteria in process of digestion (Fig. 34). These bacteria evidently are carried into the culture by the plant tissues. The majority of the amoebae contain a very few vacuoles, while a few are full of vacuoles at the posterior end and a few have no vacuoles at all. The contractile vacuole is present in some cases and absent in others. When present it is always posterior to the nucleus.

The single flagellum is about twice the length of the organism and is situated at the extreme anterior end. It ends just inside the periphery in a deeply staining granule, the blepharoplast, which is connected with the karyosome by means of a rhizoplast. This blepharoplast arises from the karyosome and appears to work its way up or into the flagellum and becomes stationed at the anterior end. During division it is first to divide (Fig. 33).

Under certain environmental conditions the flagellum is pulled back into the organism. This has been observed in living material, and the indications are that the contents of the flagellum and blepharoplast are returned to the karyosome. The flagellates feed in the same manner as the amoeba. Bacteria are always taken in at the posterior region, where they are digested in vacuoles.

Binary fission: The first step in binary fission is generally the division of the blepharoplast, although sometimes the development of the new flagellum precedes it. Next, the nucleus divides promitotically, and last of all the cytoplasm divides longitudinally. Sometimes when conditions are favorable, the cytoplasmic division at the anterior end begins before the nuclear division and proceeds rapidly posteriorly. Later stages of division give

the appearance of an elongated organism with an anterior and a posterior flagellum (Figs. 39–41). Gilbert (1929) maintains this latter stage is a contamination, but the writer has picked up a dozen or more of these bipolar organisms and has put them in an ice box. They soon changed into the amoeboid stage, and the nucleus underwent division, forming two amoebae.

From observation made during the course of the investigation, it is apparent that the flagellates occur more commonly in the cultures than the amoebae. In a fresh culture made from spore cysts amoebae usually appear, but as the culture grows older, the flagellates become more and more numerous.

Most of the amoebae in a culture can be transformed into flagellates in two hours by raising the temperature. A little over an hour after a preparation has been made from a cooled culture, contracting amoebae with developing flagella are found in different places on the cover glass. These will be found to be more or less free from the substratum, and to be moving at the anterior end by a characteristic jerky movement, through a rather wide range of space. After the amoebae have been transformed into flagellates they send out and retract short pseudopodia almost continuously, and twist and turn, getting nearer all the time to the rounded-up condition. When they reach a thick, long, pyriform shape, they swim away with narrow anterior end foremost (Fig. 38).

In changing from the flagellate to the amoeboid stage, the organisms settle to the bottom, develop pseudopodia, retract their flagella and assume the typical amoeboid form (Fig. 4). This change can be induced by lowering the temperature or by mechanical disturbance. After such stimulation the flagellates begin to whirl around until they seem exhausted, then settle to the bottom and assume the amoeboid form.

ENCYSTMENT

Encystment follows a period of rapid division, and appears to depend upon the heaviness of inoculation or

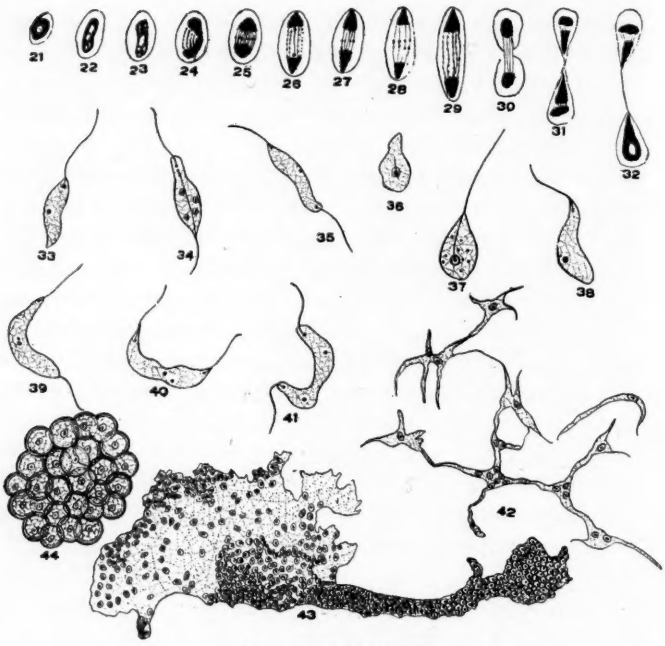
upon the relative number of amoebae in the culture. The onset of the process is characterized by a cessation of division, as only an occasional division figure is found in preparations from cultures in which most of the individuals are encysting (Figs. 10–14).

Organisms undergoing encystment are characterized by the entire absence of food vacuoles, by a rounding up of the cytoplasm and by enlargement of the contractile vacuole with an increase in the rate of its pulsation (Fig. 12). The protoplasm becomes dense, the contractile vacuole disappears, and a thin wall is formed around the organism. Later a second wall is formed within this, which is much thicker and contains no visible opening. In some encystments a sporoblast is found filled with the parasite, Calkinsi. Under proper conditions, such as the addition of fresh culture medium, these parasites appear to be similar to the dots seen in the disease plant nuclei, and chloroplasts of invaded tobacco tissues (Jones, 1926).

Plasmodium

During early spring, April and May, in the vicinity of New York City, the cytoplasm of the amoeba seems to undergo a change. It becomes more stringy and loses the limax type shape. The entire absence of food and contractile vacuoles and less differentiation between the ectoplasm and endoplasm are noticeable. The karyosome destains more readily in the stringy form, while the cytoplasm destains more readily in the limax form. The amoebae begin to move towards a common center and fuse to form a plasmodium. The writer observed the formation of a plasmodium in culture and made prepared slides to verify the procedure (Fig. 18). It is impossible to say what determines the size of the plasmodium, but the author has examined sixty-two plasmodia and they all seemed to be the same size, although each was made up of hundreds of amoebae.

Cienkowski was the first person to observe the fusion of amoebae to form a plasmodium. His description is as



Figs. 21–32. ×3000
" *33–44.* ×2000

PLATE III

FIGS. 21 to 32. Different stages in division of nuclei from a plasmodium.

FIG. 21. Prophase of nucleus in plasmodium with elongated, vacuolated karyosome.

" 22. Vacuolated, dumb-bell shape karyosome.

" 23. Same as above.

" 24. Spindle turned showing chromatic polar mass connected on one side. Spindle fibers with chromatin granules between the two ends.

" 25. Spindle of late prophase.

" 26. Early anaphase, polar caps, centrioles in centrodesmose and spindle.

" 27. Spindle at the beginning of the metaphase, showing indications of polar caps, equatorial plate, centrioles and central spindle.

" 28. Early anaphase, one distinct polar cap, all chromatin granular, central spindle.

" 29. Same as above.

" 30. Late anaphase.

" 31. Same.

" 32. Telophase.

" 33. Beginning of division of flagellate, showing division of blepharoplast.

follows: "Fusion of the Myxamoeba present occurred in a particular area drawn together into groups becoming endowed, apparently, with the power of mutual attraction, and the groups, once formed, acting as centers to which neighboring amoebulae scattered through the water converge (Fig. 42), and a complete fusion of the protoplasm occurs."

As soon as the fusion of the amoebae is complete, the plasmodium moves like a huge amoeba. The lines of flow are generally in one direction, but the course of the plasmodium may be changed. This plasmodium is different from other plasmodia that the writer has studied in that it does not take in any food during the plasmodial stage nor does it have the rhythmic flow that one often observes in the myxomycetes.

The ectosarc of the plasmodium is a very clear liquid of unequal thickness over different parts of the plasmodium and is generally abundant at the advancing margin. The nucleus of each amoeba that enters into the formation of the plasmodium retains its individuality. The majority of the nuclei in the plasmodium undergo division at the same time. Division occurs promitotically, the same as found in the amoeboid and flagellate forms (Figs.

" 34. Complete division of blepharoplast and flagellum, showing one daughter flagellum in active motion, other trailing it.

" 35. Later stage in cytoplasmic division, with anterior ends of the two individuals pulled apart, giving appearance of an elongated individual with flagellum at each end.

" 36. Amoeba changing into flagellate stage, showing outgrowth of blepharoplast, from karyosome of nucleus.

" 37. Flagellate stage showing completed flagellum connected with karyosome by means of rhizoplast.

" 38. Flagellate showing completed flagellum ending in blepharoplast.

" 39. Two flagellates with anterior ends of daughter individuals separated showing beginning of nuclear division.

FIGS. 40 and 41. Later stage in division, showing completed nuclear division and cytoplasm constricting.

FIG. 42. Amoebae fusing to form a plasmodium.

" 43. Plasmodium with hundreds of nuclei, flowing like a huge amoeba. Most of the nuclei are located at posterior end.

" 44. Cyst wall formed around each nucleus in the plasmodium which will give rise to limax type of amoeba on excystation.

19, 20). This seems to be the first time that nuclear division in a plasmodium has been described as promitotic (Figs. 21–32). The spindles are not parallel. The writer has been unable to count the number of chromosomes in the nuclei of the plasmodium. The nuclei become smaller and smaller as they undergo rapid division.

Wherry (1913) in working with the same species figures individual amoebae with two to four nuclei, all in coincident mitosis, and individuals with ten or more nuclei. Wilson (1916) believes the multinucleated condition comes from cases in which the cytoplasm failed to divide after mitosis.

The writer is convinced that the plasmodial stage is formed not for nuclear fusion but for the purpose of adding new life to the nuclei by rejuvenating the chromatin while it is in the cytoplasm. When the amoebae are coming together to form the plasmodium the karyosome and nucleus indicate lack of balance or disturbance of this normal ratio as a result of a possible prolonged vegetative activity. The chromatin final absorption before spore formation suggests the restoration of this balance.

Cyst spores are formed in the plasmodium. Just before these spores are formed, the cytoplasm of the plasmodium becomes clearer and more granular. Cyst walls then form around each nucleus, and thus a cyst spore is produced. Excystation takes place immediately, providing sufficient food is present. A limax type amoeba is formed from this cyst. The amoebae formed from the cysts may be changed from flagellates to amoebae and *vice versa,* depending on temperature.

So far, it has not been possible to culture the organism *Naegleria gruberi* free of bacteria. If cultures are entirely free, the organism does not live long. The bacteria seem to be a part of its food, and their relative abundance determines, to a great extent, the state in which the organism occurs in culture. Thus when bacteria become abundant again, the organism excysts. In an earlier paper

(Jones), the writer named this organism, which has been called *Naegleria gruberi, Plasmodiophora tobaci.* It is not a plasmodiophora, however, since it differs from that group (1) in producing a plasmodium, (2) in the method of nuclear division, (3) in the method of spore formation and (4) the fact that it is not parasitic on plants.

Summary

Soil amoeba, usually called *Naegleria gruberi,* coalesce in early spring to form a plasmodium. Before fusion, the amoebae undergo a cytoplastic change. The cytoplasm becomes filled with chromatin. The ectosarc can not be differentiated from the endosarc. Instead of blunt pseudopodia, the amoebae are found to have long stringy pointed pseudopodia without a definite shape. After fusion, the nuclei undergo rapid division by promitosis, identical to those in the amoeba stage. The majority of nuclei divide at the same time, but the spindles are not parallel. After division, a cyst wall is generally formed around each nucleus, and on germination each cyst gives rise to an amoeba or a flagellate (depending on food and temperature) such as were found in the original culture. No food is taken into the organism during the plasmodial stage.

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GROWTH AND LIFE SPAN OF THE FIELD MOUSE

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THE data recorded in this paper are based on a four-year study of the field mouse, *Microtus pennsylvanicus pennsylvanicus* (Ord) at Ithaca, New York. More than 4,000 mice have been examined from the spring of 1933 until the fall of 1936. This report is part of a study conducted to determine the bionomics of cyclic fluctuations in rodents.

That microtine rodents grow rapidly and attain sexual maturity at an early age has long been known. Bailey (1924) has shown that *Microtus* females mate with older males when but 25 days old, and the young males becoming fecund when only 45 days old. It is further stated that the young, after the first few days, gain about one gram a day until over half grown. This rapid increase has been established for the larger *Microtus californicus,* which, according to Selle (1928), attains an average weight of 50 grams when 45 days old. The young average 2.7 grams at birth. The studies of Selle further indicated that most of the growth is made during the first two months, after which the rate of growth is slower. All the foregoing data were based on captive animals.

To determine if laboratory conditions had material effect on rate of breeding and growth, field studies were inaugurated, so that a contrast might be made of such with controlled conditions.

Mice were readily taken in Sherman metal box traps, baited with oat flakes or small pieces of apple. Such traps take the mice alive and unharmed. The mice were etherized, marked by incising one or more toes, measured, weighed, the condition of the external genitalia and teats noted and then released. A large number of nest young were marked. After disturbance of the nest and young,

the mother invariably removes them to a new nest. After weaning (11 to 12 days) the young mice become active and are easily retaken.

In such a study, it is desirable to establish a weight, measurement or series of such at which sexually mature individuals can be separated from those which are unable to breed. While no mean length could be established by which to separate the fecund animals from others, it was found that weight is an indicator of this condition. Following methods similar to those outlined in the suggestive paper of Baker (1930), it was discovered that mice over 25 grams were usually capable of reproduction, while those under that weight were normally sexually immature. The basis for this reasoning will be found in Fig. 1.

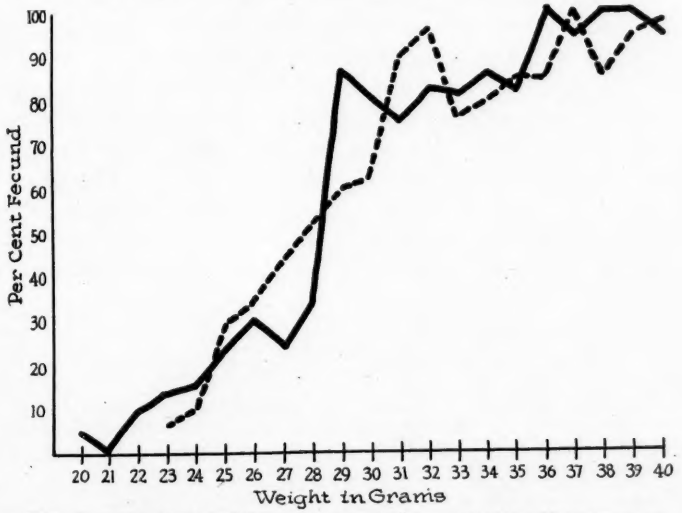

FIG. 1. Weight at which sexual maturity is reached in *Microtus pennsylvanicus*. The unbroken line indicates males; note that only 5–15 per cent. are fecund under 25 grams. The females (broken line) over 25 grams show a large per cent. of breeding mice.

It will be noted that few males weighing less than 25 grams were fecund, and that in only a few instances did a female at this weight show indications of having bred.

In order that fecundity be established in the male, the tail of the epididymis was crushed and smears of such examined microscopically. If sperms were abundant the animal was recorded as fecund. If but a few were present, and the epididymis was too small to indicate the tubules macroscopically, the animal was listed as not fecund. The size of the vesiculae are likewise an index to the functioning of the sex hormone. The size of the testes further indicate the presence or absence of sperm. If smaller than 8 by 4 mm, sperm were never found in the epididymis. Large testes in old males frequently measure 20 by 12 mm.

Sexual maturity in the female may be determined by the presence of embryos, corpora lutea or evidence of previous lactation. Perforation of the vulva is no criterion, for such is often tightly closed in multiparous individuals which have not bred for a month or longer.

The data in Fig. 1 are based on an examination of 671 males between 20 and 60 grams and 608 females between 20 and 45 grams. A number of males were taken in excess of 60 grams, the largest weighing 71 grams. To determine the vital weight of gravid females, embryos were removed before weighing. All females more than 45 grams in weight had bred; the largest collected was 56 grams. Inasmuch as it is extremely unusual to secure mice over 40 grams which have not, at some time, been fecund, all over this weight were omitted from the calculations in Fig. 1.

While nest young are readily secured, it seemed desirable to raise a number in captivity, marking and releasing them when weaned. Captive mice were housed in large metal inclosures during 1934, 1935 and 1936. They were supplied with abundant dirt and a variety of natural foods and water. Exercise wheels were available. The mice were kept in a fruit cellar, which allowed for humidity approximating field conditions. Diffuse light from a window struck the pens during the day.

Growth records are available on 24 litters totaling 152 young, an average of 6.3 young per litter. In the late

spring and summer of 1936, following a period of maximum density, the reduced populations produced litters considerable less than this, the average number being nearer 4. The average birth weight of these was 2.07 grams, the extremes being 1.6 and 2.9 grams. In some instances the young had nursed when first weighed. The mean weight is reasonably accurate, for a series of full-term embryos (77) closely approximate this figure (1.92 grams).

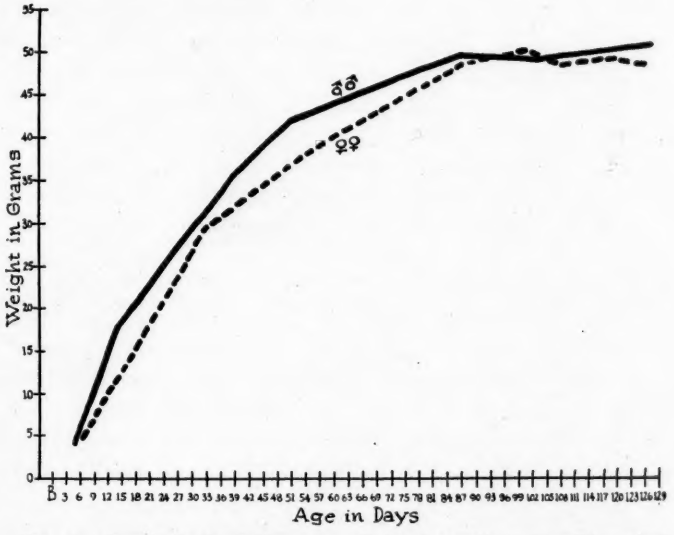

FIG. 2. Growth in the field mouse. Rate of growth in 34 wild marked males and 31 wild marked females, supplemented with growth data of captive mice from 1 to 14 days old. Mature size is attained in 12 weeks.

Fig. 2 is based essentially on data collected from mice growing under natural conditions. It is the average growth rate of 34 males and 31 females, trapped repeatedly during the first four months of their lives. During the first 15 days the figures are supplemented with growth data obtained from young captives, which, incidentally, agree surprisingly well with that obtained from wild nest young. It well illustrates the rapid growth rate until the twelfth week, when the steady increase is retarded.

At this period we may say the mice are nearly full grown.

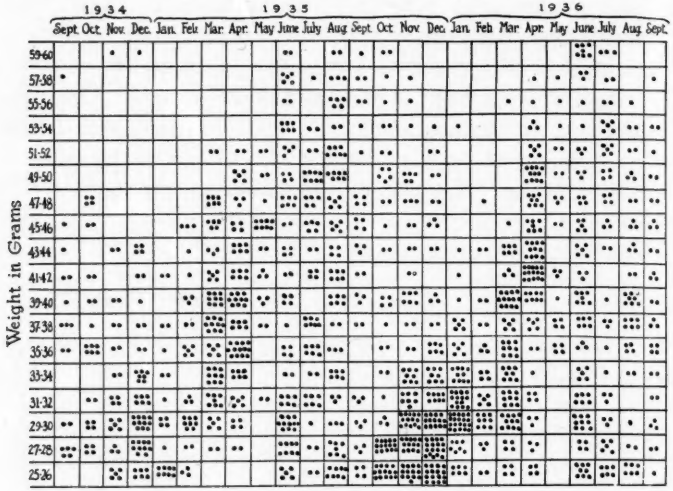

FIG. 3. Body weights of sexually mature male field mice in relation to seasonal captures. There is a notable lack of mice in excess of 45 grams during the winter months.

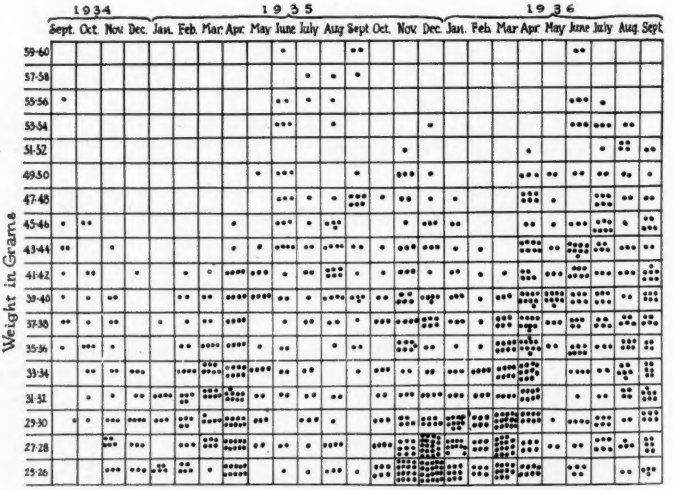

FIG. 4. Body weights of sexually mature female field mice in relation to seasonal captures. Proportionally few mice over 45 grams were taken during the winter months.

A study of Fig. 3 and Fig. 4 at once indicates the almost total absence of large adult mice during the winter months. This paucity of heavy adult specimens can not be attributed to seasonal habits, which would make large adults more secretive. Furthermore, there is little indication of a weight reduction in individuals during the winter. Food is usually abundant, and all winter specimens have been notably robust individuals, well supplied with fat. The only plausible explanation is that the older mice (those which have attained a weight in excess of 45 grams) die off, the survivors being born during the late summer or early fall of the preceding year. It is conceivable that some large individuals live through the winter. These data tend to support the view of Baker and Ranson (1933), who found the fully grown males and most of the females (*Microtus agrestis*) do not live over to the next year. Their observations point to death, presumably of old age, during or shortly prior to October. The British mice, however, appear to differ from the American *Microtus* in their reproductive traits. While our mice commence to breed at an exceedingly early age, no such precocity is apparent in *M. agrestis*. Furthermore, the British mice have a very well-defined breeding season, commencing in mid-March and ending in late September. In eastern United States the length of the breeding season is determined by abundance of mice. When few are present, as during the low of a cycle, the breeding season is foreshortened. When at the height of the cycle, with optimum densities prevailing, reproduction, although curtailed in the winter, continues throughout the year.

Data have been introduced which are suggestive of a short life span in *Microtus pennsylvanicus*. The adult mice ostensibly die in the late winter, when less than a year old. In the case of those born late in the fall, the mice perhaps overwinter, but these individuals die when slightly more than one year old.

If longevity in other small mammals is considered, it is found that those which have a normal life span in excess

of two years are considerably less active than *Microtus*, nor are they so prolific.

Dice (1933) kept captive *Peromyscus* males alive for 5, 7 and 8 years, while a female lived for 5 years, 3½ months. Captive deer mice were kept by Sumner (1922) from 3 to 5 years, venerable females bearing young when 3 years old. In central New York, deer mice customarily commence breeding during mid-March, and lactating females are not uncommon in late October. A notable paucity of gravid females is found in late summer. It is extremely unlikely that more than five or six litters are produced in a season. Hence the reproductive burden is not as prolonged as in *Microtus*. Again, activity of deer mice is restricted largely to the night, in contrast to activity at all hours by *Microtus*. Thus metabolism is geared to a high pitch in the more active field mice. Such a combination would presumably hasten senility and consequent death.

The rat (*Rattus norvegicus*) according to the extensive observations of Slonaker (1912) is to be regarded very old at the age of three years (*i.e.*, comparable with a man of 90), while it is stated that the female commonly becomes sterile at the age of 15 to 18 months. We find this animal more prolific than *Peromyscus* and less so than *Microtus*. In New York the rat breeds throughout the year, but the rate of breeding is less than that to be found in *Microtus*. Furthermore, lactation lengthens the gestation period in the rat, thus limiting the number of litters per year. Such a condition does not prevail with microtines, which normally wean the young long before the next litter has appeared.

There are thus three factors which appear to operate to produce a short life span in the field mouse. These are (a) attainment of sexual maturity at an uncommonly early age (b) extreme prolificness and (c) little cessation of activity in its search for food.

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SHORTER ARTICLES AND DISCUSSION

CYNIPS AND LYMANTRIA

In a recently published bulky paper, the second in the series, A. C. Kinsey reports upon his further taxonomic work in the genus Cynips, which has given him a rather complete knowledge of the geographical variation in this form. To the detailed descriptions of the new material he adds a general discussion upon the topic which has furnished the title of his paper, "The Origin of Higher Categories in Cynips." This general part contains, also, a chapter on the correlation of taxonomy and genetics in which my work on Lymantria is singled out for (unnecessarily aggressive) criticism. The purpose of this anticritique is to show that the actual results, as far as the taxonomic side is concerned, are to all purposes identical in both cases, which the critic overlooked because he based his criticisms on short versions rather than on a study of the original publications; further, that Kinsey's claim that his work sheds light upon the origin of higher categories is unfounded; that the results of his studies do not go beyond what has been known in other groups; finally, that no facts have been brought to light which will force geneticists to change their ideas.

Kinsey, in opposition to the majority of taxonomists, calls the lowest category which can be distinguished a species. Groups of such species, which groups may be distinguished more or less artificially, he calls complexes. These, again, combine into higher categories, the subgenera, and these together constitute the genus. All these "higher categories" above the species are considered as artificial, distinguished only for convenience sake. In this case Cynips is the genus, which is divided into six subgenera (most important: Atrusca, Philonix, Acraspis). These again, are composed of altogether twenty-six complexes with a varying number of species each. All these forms have been followed through the entire range of the groups. The decisive results are the following: The majority of the "species" may be arranged in chains, each two members of which are as near to each other as the geographic races or subspecies in usual taxonomic nomenclature. Thus the subgenus Atrusca contains 43 species which can be arranged in a continuous chain, and there are 76 of the combined subgenera Philonix, Acraspis, Sphaeroterus. Thus at some point

in these chains the transition from one subgenus into another is not different from the typical transition from one species to another. The ends of these chains, however, may be as diverse as "genera" and be described as such, if the links are not known. The whole group (Cynips) may then be described as one branched chain of species; the major divergent branches are the subgenera, their offshoots and stems are the different complexes, and the different nodes are the species. If no secondary isolation has taken place, all these links are continuous and connected and the phylogenetic tree is identical with this actually existing tree of species.

Before adding some details to this general picture we may compare it with our knowledge of other cases and also of Lymantria. We may mention at the outset that in Kinsey's work indeed a very elaborate body of material has been brought together, surpassing in the number of almost 200 units (species) and the manifold branchings of the chain of forms other known cases. This is due to the fortunate choice of material, which lends itself to a considerable amount of isolation and therefore restriction to small areas. Kinsey quite aptly compares these forms to an island fauna, the mountain tops and isolates of growth of the host (white oak) being comparable to islands. This considerable diversification of the forms makes it possible to follow the chains of forms in different branchings towards a number of extreme ends of these branches. Thus a more complicated picture in detail has been worked out than probably in most other cases thus far studied. But the decisive point is that the results do in no way differ in principle from those of the mammalogists, ornithologists, conchologists, lepidopterologists, who have worked out what is called the Rassenkreise of species. Kinsey mentions this similarity briefly, but does not point out clearly how nearly related his results are to those of all those modern taxonomists. These hold that the unit is a species, which consists of a chain of geographic races or subspecies, all different, fertile inter se, all occupying a definite not overlapping area and which at the end of a chain might be as different as typical species. In many of these cases it turned out that what had been described as distinct species were found to be only links in, or ends of, a chain, when all intervening forms became known. This is now exactly what Kinsey has found, maybe in a more elaborate example, though it is obscured by his arbitrary nomenclature and not plainly acknowledged. In the nomenclature of the majority of modern taxonomists he has

studied a Rassenkreis of a single species, formed of 170 odd geographic varieties or subspecies. For purposes of description he has divided these into two categorical subgroups (his subgenera and complexes). The actual results coincide completely with those of the workers on Rassenkreis (see the reviews by Rensch), though his material is quantitatively more elaborate. This now disposes completely of his claim to have made a contribution to the origin of higher categories, a claim which is based entirely upon a new nomenclature and not upon a new type of facts. Like all the other workers on Rassenkreise he has shown that what usually is called a species (called by him a genus) is in fact a chain of nearly related races, the extreme links of which might be very different. The evolutionary problem now stands just where it stood before: Are the extreme members of the chains the beginnings of new species (not species in Kinsey's nomenclature) as most taxonomists believe (exhaustive discussion in the book by Rensch); or is this whole variation occurring only within that unit, here called the Rassenkreis, without major evolutionary significance, as the author holds? Kinsey's work does not furnish any material beyond the type already known which could change the balance of the scales or which would allow resumption of the discussion on the basis of a new type of facts.

Now for Lymantria, which Kinsey classes with Drosophila as a laboratory animal. In mentioning within a paragraph directed against the author's work the very few studies "which have been done checking the experimental results by taxonomic work" he does not include Lymantria, showing that it is unknown to him that my experimental work proceeded hand in hand with more elaborate taxonomic work than ever done in a species of Lepidoptera. Repeatedly he speaks of the few species which were included in my work. If he had read the original, unfortunately rather bulky work instead of a short review, he would have realized that here again only a difference in nomenclature is involved. Actually the variation of *Lymantria dispar* was covered over the entire range of the species, though more work was done upon the more interesting Eastern Asiatic forms than upon the Eurasian ones. If I had cared to use a nomenclature like Kinsey's, I should have called the gipsy-moth the genus Lymantria and the nearly related nun-moth, for example, the genus Porthetria. Between both, inhabiting the same area, no connecting link exists, though both are thoroughly known. Within the genus Lymantria I

should have distinguished a number of subgenera, *e.g.*, the European and Russian weak races as one, the South European races as another, the very different Hokkaido group as a third, and so on, of course, giving names to all. Then I would have found complexes: the actual groupings which I used, *e.g.*, the North Japanese strong races, the Korean half-weak races, etc., correspond exactly to Kinsey's complexes. Within these groups I found, again, subgroups, *e.g.*, within the strong "complex" of Northern Japan special groups in the mountainous district, in the Gifu plain, etc. And within these groups I might finally have described as many "species" in Kinsey's sense as I wished, as I have emphasized over and over again that practically each locality which I had covered in my extensive field work (the laboratory animal!) had a different form. The reason that I did not do this was that a very close study of all distinctional characters, morphological, developmental, ecological, physiological, which could be found (according to Kinsey I would better study more characters) revealed the fact that the transgressive variability of the individual traits is such that it is safer to deal with groups of the lowest units than to try the impossible, in Lymantria of course, of working out those elementary units which Kinsey calls species.

I realize, of course, how difficult it is for a taxonomist to deal with such a description as I have used in Lymantria, where I failed completely to give Latin names to the subgroups and races which I found. The reason is that it was found that most of the distinctive characters used by taxonomists in this case are not important for classification (see the psychological experiment described in Goldschmidt, VII, p. 606). Names are, however, of no use if they do not serve for identification, which in this case is not possible from pinned specimens.

It would be easy to point out that the Rassenkreis of the gipsy-moth shows all those additional features which Kinsey emphasizes in Cynips. There are the continuous chains, there are the unconnected isolates described by taxonomists as different species (Hokkaido), there are the very different ends of a series which connect somewhere with another chain (Korea-Manchuria). But also all those details are found, similarly in both cases, which thus far have not been mentioned: the presence of simple Mendelian mutants within one race, the common possession of one trait by different members of the chain, differing in others; the sudden appearance of a decisive new trait in a chain, which in other traits

is continuous (*e.g.*, the frontier between strong and neutral races in Middle Japan); the presence of "mutant" differences within a race which otherwise characterizes two different races (*e.g.*, wing color of Hokkaido and Aomori males). It is not needed to go into further detail, which will be found in the original papers and some of which may also be gathered from a more elaborate discussion (1935) which Kinsey also missed.

I shall not try to answer in detail the polemical part of Kinsey's paper, in which many points may be found which are not very fortunate. Two examples will suffice. On page 66 Kinsey writes:

> Such limitations are hardly being followed by those who, exercising a very scientific scepticism of all taxonomic data, are still ready to offer "speculations" on the possibilities of "directed orthogenetic evolution" proceeding from "hopeful monsters" adapted to "some empty environmental niche"—to summarize Goldschmidt's own method of attacking the problem of species.

Thus the general reader who is not acquainted with genetic literature is led to believe that a few timely speculations offered on the occasion of a lecture on a general topic (in addition ridiculed by taking a few catchwords out of their logical context) have been my only contribution to the subject. He is not informed that my factual contribution to the problem constitutes thus far the most elaborate and most complete analysis of a single Rassenkreis made, using the most diversified methods of observational and experimental attack, including a complete taxonomic analysis of the material collected by myself in the field (including the discovery of almost all different races), checked by experimental breeding under constant conditions, as well as the genetical analysis, as far as it could be pushed. He is not informed that about 600 printed pages were filled with nothing but new facts without hardly a single speculation and that these facts, as far as the taxonomic analysis is concerned, which is only a small part of this work, are in no important point different from the facts found by Kinsey, of course barring the difference in nomenclature and in the ecology of a gall wasp and a moth. He is not informed that the speculations mentioned in the above phrase are partly classic conceptions of evolutionary thought (preadaptation) and further not that these speculations applied to large differences, say, of real generic or family nature, which are not accessible to genetic analysis and that they are completely independent of the work done on the problem of geographic variation.

The second point is the following: On page 66 Kinsey writes:

To ignore all taxonomic studies because they are not experimentally analyzed seems an undue limitation of the possible significance of genetics to the lives of Drosophila, to gipsy-moths and to a relatively few other organisms.

Here are a few quotations from my papers, demonstrating how I ignore taxonomic work:

If we follow the work of modern taxonomy, *i.e.*, of the workers who know best the actual material in nature and who by virtue of this knowledge are best fitted (next to paleontologists) to judge the results of evolution (though not the methods of evolution) we find. . . .

Then follows a description of the Rassenkreis theory, containing also a statement that I had better chance to follow taxonomic work than most geneticists have (Goldschmidt, 1933, p. 173). Further (Goldschmidt, 1935, p. 12):

It seems to me that the decision in both problems (geographic variation and adaptation) rests with genetics. This is not the expression of one-sided overestimation of my own field of research. Actually my own work was started, twenty-five years ago, on the basis of my knowledge of, and my admiration for, the results of the taxonomists, which then began to crystallize into the Rassenkreis theory. But I realized that even in the best known groups in which large series of individuals have been studied, the birds (Kleinschmidt, Hartert, Stresemann, A. O.), the rodents (especially Grinnell), the Lepidoptera (Jordan, Forbes), taxonomic work can not deal with some decisive difficulties: (1) It can not be decided whether a form typical for a given area is essentially a non-hereditary modification or a hereditarily different form. Only occasionally is a decision possible (Stresemann). (2) As a rule it can not be decided whether the differential characters which alone are accessible to the taxonomist are adaptational or not. (3) All physiological characters, as a rule, are excluded, though they must be considered *a priori* most liable to be adaptational. (4) Larval characters are completely or almost completely excluded and very frequently also the ecological traits, which may be of decisive value, *e.g.*, in insects. (5) If an intermediate form occurs between two areas it can not be decided whether this is a consequence of hybridizing, or a series of multiple alleles with intermediate effects of each member, or of a modification, or of the combined effect of different genes; only occasionally segregation of forms in such an area may bring the decision (without experiment). (6) Fertility assumed to exist between the members of a Rassenkreis and sterility (at the ends of the chain) only rarely are actually proven. (7) As long as the genetical nature of the difference between two members of a Rassenkreis is not known, conclusions regarding the origin of such differences are unwarranted.

These two quotations may suffice. They are contained in the two only papers in which I have discussed the results, all other papers presenting only the facts. It is of no use to extend the

argument beyond saying that Kinsey's method of quotation and criticism can hardly be called a commendable scientific method.

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THE PHYSIOLOGY OF HETEROSIS

IN a recent number of *Genetics* East published a pronouncement on heterosis (1936). The substance of his contribution was an account of the occurrence of heterosis in amphidiploids and a criticism of what he calls "Ashby's physiological theory of heterosis," which he concludes is "unsound in all its essential features" and which is "destroyed utterly" by the general body of information on heterosis (p. 380). Since East's conclusions are likely to carry weight wherever genetics is studied and since East has invoked the present writer's data to support his own conclusions (pp. 380, 389), it is necessary to examine the validity of his argumentation.

Ashby has not published any theory of heterosis. There is accordingly no statement of the theory in East's paper. The writer examined size-heterosis in certain strains of maize hybrids, from germination to the onset of flowering, and concluded that "hybrid vigour *in these strains* is nothing more than the maintenance of an initial advantage in embryo size" (1932, p. 1031). Far from applying the discovery beyond the specific data, Ashby stated (p. 1030):

> The experiments reported in this paper have been confined to a few strains of maize, and one is not justified in extending these conclusions further, even

to other varieties of maize. For, as will be pointed out below, the mechanism of hybrid vigour may differ for different plants.

Despite this warning East has assumed that Ashby's conclusion is of general application, and he has then proceeded to confute his own assumption. If the assumption had ever been made, the writer would be the first to condemn it. East implies also (1936, p. 375–6) that the writer's data on maize constitute an attack on Jones's hypothesis of heterosis, whereas the writer stated (1932, p. 1026):

> The data assembled in this paper are not at variance with the mendelian hypothesis of hybrid vigour, though they require certain qualifications of that hypothesis.

And later, concerning these qualifications:

> It must not be implied that this hypothesis is of general application to the inheritance of growth characters; it merely offers an explanation for the data offered in this paper. . . . In certain circumstances the efficiency index of a hybrid may not be identical with that of either parent. . . . A combination of parental characters which eliminates in the hybrid a deficiency factor, may result in an increased efficiency index.

The analysis has recently been extended to tomatoes (Ashby, 1937) with substantially the same result; and the sum total of the present writer's conclusions may be considered as a statement of what heterosis is *not*.

East's criticisms may be dismissed, therefore, with the remark that they *might* have been appropriate if any one had claimed that Ashby's conclusions were a general solution of the problem of heterosis. More serious difficulties arise, however, in accepting East's manipulation of the facts of heterosis. He postulates (1936, p. 388) that the effect of hybrid vigor is "comparable to the effect on a plant of the addition of a balanced fertiliser to the soil"; and (p. 389) that "heterosis is ordinarily manifested by a rise in the efficiency index, in the sense with which Ashby used the term."[1] This, he says, "is what is shown so nicely by Ashby's logarithmic curves." A glance at the illustrations of Ashby's papers (1930, 1932, 1937) will show that an increase in the efficiency index of hybrids *does not occur in a single instance*. So far as the writer is aware, there is no case in the literature of genetics of heterosis being attributable to a continued higher

[1] On page 376 (1936) East states, quite correctly, that the efficiency index is the "angle of slope of the logarithmic curve of growth."

efficiency index in the hybrid; although, as the writer pointed out in 1932, such cases are likely to occur, even though no occurrence has yet been reported. Unless East can publish data in support of his statements quoted above, they must be unconditionally rejected. They are completely contradicted by Ashby's own data, which is the only evidence he brings to support them.[2]

On page 380 of his paper East makes the same mistake. He states: "Heterosis, therefore, is not simply a manifestation of an initial advantage in embryo size. . . . It is a genetic effect on the organism as a whole, . . . as even Ashby's mathematical treatment of his results has demonstrated." Reference to the writer's papers will show that there is no evidence for this statement, and that analysis of the data led to the opposite conclusion!

It is interesting that the figure published by East and Jones in their excellent summary (1919, p. 152), as an example of heterosis, provides a striking parallel to the writer's own work. Data on height (although unsatisfactory as a measure of growth in maize) are given for inbred parents and an F_1 population. There is considerable height-heterosis—the hybrid was nearly three feet taller than the bigger parent after 90 days—yet the *ratio* of the heights of the F_1 to the parent Pl–9 was 1.40 at the first sampling and 1.45 at the last. The relative growth rates of the parents were 0.0188 and 0.0190; and of the hybrid 0.0192—an increase of 1.04 per cent., and judging from the variance usual in homozygous populations of maize, quite insignificant! During the experiment which these data record, the hybrid, despite its heterosis, was *not growing in length relatively any faster than its parents.* Jones' data, therefore, although they are meager and can not be properly analyzed, lead to the same conclusion as Ashby's, namely, that the advantage in size of the hybrid was present *before the experiment began.* This means one of two things: (1) that the growth rate of the hybrid was greater from germination to the time of the first sampling or (2) that the initial size of primordia in the hybrid embryo was greater. What little evidence the writer has collected points to the second of these explanations as the proximal cause of size-heterosis in certain maize and tomato plants. Sprague (1936) has recently published evidence from maize which favors the first explanation, though he too finds no greater relative growth rate associated with heterosis over the first 45 days of growth.

[2] An experiment by Lindstrom (1935), purporting to test this point, has already been discussed (Ashby, 1936).

As regards embryo size, East fails to realize that it is the primordia in the embryo which determine subsequent size, and that the size of non-meristematic parts (cotyledons, scutellum, endosperm, testa) are of secondary importance, if indeed they have any significance at all. He admits that apart from one example from his own work there are no observations recorded in the literature "where the component parts of the seed have been separated" in estimating the effect of seed size on plant size. Unfortunately in East's own example the primordia and scutella are not separated, nor is anything stated about the final size to which the embryos he measures will grow; so the data have no bearing on the present problem. The data are worth reproducing here, however, as an excellent example of the lack of correlation between primordial size and grain weight (Table 1). The writer's own work corroborates this; he has recorded (1932, p. 1014) grains with weights of 250 and 220 mg, respectively, whose embryos weighed 2.66 and 3.57 mg. It is obvious from an inspection of East's data that in no sense is grain weight a measure of embryo size. This is true even in exalbuminous seeds like the tomato, where as much as 75 per cent. of the seed weight is testa, and no measure of the primordial size at all.

TABLE 1

DATA AS TO GRAIN WEIGHT AND EMBRYO WEIGHT IN VARIOUS STRAINS AND CROSSES OF MAIZE, TAKEN FROM EAST AND JONES (1920, TABLE XV, PAGE 568). IF GRAIN WEIGHT WERE A MEASURE OF EMBRYO SIZE THE RATIOS IN COLUMN THREE SHOULD BE CONSTANT. THE RANGE OF VARIATION IS IN FACT 4.3 PER CENT., WHICH IS ABOUT 50 PER CENT. OF THE MEAN!

Embryo wt.	Total wt.	Ratio	Embryo wt.	Total wt.	Ratio
2.68	26.63	10.1	2.57	31.94	8.1
2.63	34.91	7.5	2.10	29.02	7.2
3.04	29.75	10.2	3.10	33.04	9.4
2.29	31.60	7.3	1.82	25.91	7.0
3.30	28.71	11.5	3.37	31.69	10.6
3.10	35.19	8.8	2.26	29.94	7.5
3.05	26.44	11.5	3.18	34.41	9.2
2.15	26.34	8.2	1.92	25.96	7.4
2.62	25.72	10.2	3.33	34.18	9.7
4.83	54.33	8.9	3.64	49.60	7.3

The only published data as to embryo weight in relation to subsequent size seem to be those of Ashby, therefore. In these data primordial size is clearly the major cause of size-heterosis; but judgment as to its importance for heterosis in general must be suspended until further data are published. East, however, prefers to reach a conclusion at once, from sundry examples of the inconsistency between heterosis and *seed* weight, regardless of the fact that his own data show there is no relation between seed

weight and embryo weight, and that there is ample evidence that amount of endosperm does not determine final size. By varying the nutrient supply to the inflorescence, the amount of food stored in the seed may be varied considerably; but the size of primordia in the embryo, and often the size to which the plants grow which germinate from such seed, may be unaffected. Therefore when East plants peas of "practically the same size" (1936, p. 378) and finds that they grow into plants of different sizes, it is no evidence in favor of his thesis or indeed of any thesis at all; and even when heterosis is certainly due to post-germination processes (as, for example, in the classical work of Keeble and Pellew) the fact scarcely diminishes the importance of embryo size for heterosis in some strains of maize!

The situation can be summarized as follows: to justify the assertions in East's paper three pieces of evidence are necessary:

(1) Evidence that the dependence of heterosis on embryo size *has* been applied beyond the limits claimed by Ashby.

(2) Evidence that size heterosis is accompanied by a higher efficiency index.

(3) Evidence that size heterosis is not generally preceded by larger primordia in the embryo, sufficiently larger to account for the subsequent heterosis.

These three pieces of evidence apparently do not exist in the literature, though Sprague's data (1936) indicate that in one instance the weights of hybrid embryos are no greater than the weights of the parental embryos.

Two other points call for comment. East mentions that heterosis is maintained in hybrids through vegetative propagation (1936, p. 380). This is an important point, and has been receiving attention. The probable explanation is that the leaf and stem primordia in such hybrids are bigger. The importance of primordial size in the determination of organ size was stressed by Sinnott fifteen years ago (1921).

The second point concerns heterosis among animals. East cites data of Roberts and Laibile on a cross in pigs, which he considers "destructive" to the writer's "thesis" (1936, p. 379). Unfortunately he omits the data essential to an understanding of the problem, namely, the birth weight of the cross and inbred pigs. In calculating ratios of birth weights one can use only females, for no males survived in the Duroc Jerseys. The rele-

vant data from this experiment are summarized in Table 2; from which it appears that the ratio of weights after six months was slightly *less* than the ratios at birth. The difference is not significant, of course. The data show nicely that the hybrids were 25 per cent. heavier at birth, and after six months, despite considerable heterosis, the hybrids were still only 23 per cent. heavier. In this instance there was in fact *no difference* in efficiency index between the hybrids and the inbred strains. Size heterosis in the cross was nothing more than the maintenance of an initial advantage in birth weight—a striking parallel to the writer's work on maize!

TABLE 2

DATA ON WEIGHTS OF PROGENY FROM A DOUBLE MATING OF DUROC JERSEY SOW WITH (I) POLAND CHINA BOAR, AND (II) DUROC JERSEY BOAR. PRIMARY DATA FROM ROBERTS AND LAIBILE (1925). DATA FOR FEMALES ONLY; FIGURES IN POUNDS

	Duroc × Duroc	Duroc × Poland	Ratio
Birth	3.15 (mean of 3)	3.95 (mean of 2)	1.25
After 6 months	185.5 (mean of 2)	228 (mean of 2)	1.23
Efficiency index	2.770	2.761	

This advantage must have been gained somewhere between fertilization and birth, just as the advantage in some plants is gained between fertilization and the setting of seed. It simply refers the problem back to an earlier stage in the life-cycle. It is significant that Gregory and Castle have found in rabbits that some of the size differences visible in adults are already present a few hours after fertilization (1931).

The fact that the conclusions advanced by the writer for a few strains of maize and tomatoes can be inferred also from Jones' own data, and from data on animal crosses showing heterosis, indicates that Ashby's suggestions may have wider applications than he claimed for them. Since 1932 more experiments have been done on heterosis by the writer and his students, the results of which are being published. Any further discussion on the problem of heterosis is therefore better left until the fresh data are in the hands of geneticists. It is well to say in conclusion that an understanding of the physiology of heterosis, which is the aim of the writer's work, will not provide a genetical explanation of it; nor does the genetical picture of heterosis given by East offer any help in the solution of its physiology.

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POLLEN TUBE BEHAVIOR IN SELF-FERTILE, SELF-STERILE AND INTERSPECIFIC POLLINATED RESEDACEAE

THE pistils of *Reseda* species do not have the characteristic angiosperm style or stylar tissue, but only stigmatic surfaces imposed upon a "capsule like" ovary. This feature of pistil structure has raised the question as to how pollen germinates and pollen tubes develop. Mueller (1857), in a monograph of the family Resedaceae, demonstrated the presence of pollen upon the stigmatic surfaces but was unable to show the germinating pollen or pollen tubes.

The use of a pollen tube technique described by Buchholz (1931) made it possible to study pollen germination and subsequent pollen tube development. The pollen germinates upon the stigmatic surfaces and pollen tubes grow along the inner wall of the ovary through parietal placenta tissue where the attached ovules are reached and fertilized. Pollen germinates within three hours after pollination and the pollen tubes grow from point of pollen germination to the ovule in about 12 or 18 hours, depend-

ing upon conditions of temperature and humidity. These studies were carried out with the temperature ranging from 22 to 25 degrees C. Pollination, pollen tube growth and fertilization are markedly affected by external environmental conditions.

This procedure of pollen tube investigation was used in studies of self-sterile, self-fertile and interspecific pollinated *Reseda* species. Darwin (1876), in his studies upon the effects of cross- and self-fertilization, found certain plants of *Reseda odorata* to be self-sterile and others self-fertile. A self-sterile plant in the cultures of *Reseda odorata*, when selfed, showed normal pollen germination and pollen tube growth. Pollen tube development is the same for the self-sterile plant as the self-fertile plant when selfed or cross-pollinated. Thus the factors involving sterility are apparently not due to a failure in pollen germination or pollen tube growth. The studies were carried out in the laboratory as well as field and greenhouse pollinations.

Interspecific pollinations showed normal pollen germination and subsequent pollen tube growth for seven species crosses made within the genus *Reseda*.

A further test of pollen viability and pollen tube behavior was made upon the styles of *Datura stramonium*. Pollen germinates upon the stigma and pollen tubes grow in the stylar tissue of *Datura* in the same manner as in the parietal tissue of *Reseda* ovaries. It is of interest to note the normal growth of pollen of *Reseda* upon styles of *Datura* since the families differ from each other in the presence of a style in the case of Solanaceae and the absence of a style in Resedaceae.

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THE VIABILITY OF TEN-YEAR-OLD DIDINIUM CYSTS (INFUSORIA)

The rapidity with which the chemical and physical properties of fresh waters may be altered through rainfall, drought and

other factors subjects their protozoan inhabitants to hazards that demand for their survival radical structural and physiological readjustments. Such readjustments usually take the form of encystment, and the factors that induce encystment, the resistance of cysts to various unfavorable conditions and the maximum length of survival in the encysted state have long interested naturalists and protozoologists. Regarding the last point, it has been found that certain protozoa can endure a remarkably long period of encystment. For example, dried cysts of the following protozoa remain viable for these lengths of time, according to the authors cited: *Spathidium spathula* and *Oxytricha* sp., four years (Dawson and Mitchell, 1929); *Colpoda cucullus,* over five years (Dawson and Hewitt, 1931); *Gastrostyla vorax,* three years (Nussbaum, 1897); *Oicomonas termo,* over five years (Noc, 1914); *Haematococcus pluvialis,* eight years (Reichenow, 1929). Cysts of the ciliate *Didinium nasutum,* which were kept sealed air-tight in a vial completely filled with infusion, were found by Mast (1917) to be alive after five years. Mast also succeeded in activating cysts of Didinium after about eight months in the dried state, but I have never been able to revive the dried cysts of this form.

The viability of Didinium cysts is of particular interest. Specialized as Didinium is for feeding almost exclusively on Paramecium and characterized by great voracity and a high fission rate (four to nine divisions daily), it requires for its continued survival in the active state enormous numbers of Paramecium. While Paramecium is one of the commonest of infusoria, such factors as stream-flow, dilution by rainfall and scarcity of bacterial food often keep its numbers well below the level required by a Didinium population. In consequence, Didinium is not a common ciliate; not only must it withstand the usual climatic adversities, but it must not unlikely survive long periods of starvation. These periods are passed in the encysted state. How long can it survive in the encysted condition? Had Mast's Didinium cysts attained the maximum in longevity? These questions attracted my particular interest some years ago in the course of a program of genetic studies on Didinium, and, with a view to answering them, the experiments here recorded were begun.

In October, 1926, twenty 25-cc vials were each half filled with timothy hay infusion from a flourishing culture of *Paramecium caudatum.* Additional paramecia were concentrated with the

centrifuge and added to the vials, until each contained an enormous number. Then, a dozen or more specimens of *Didinium nasutum* from a mass culture were introduced into each. Under these conditions the didinia multiplied prodigiously, and within a few days the paramecia were all consumed. Next, the didinia began to encyst on the sides and bottoms of the vials, in masses of bacterial zooglea, and on pieces of hay put into the vials for this purpose. Soon each vial contained many thousands of cysts. The level of the contained fluid was marked on each vial, and the vials were left open for a week until the bacterial action subsided. Distilled water was added in small quantities in the meantime to keep the fluid level constant. This was done to prevent the many cysts that were attached to the sides of the vial near the water line from becoming dry, for the cysts in my experience do not withstand desiccation well. Hence, all cysts were immersed continuously in hay infusion throughout this entire experiment. Finally, the vials were tightly closed with cork stoppers, the stoppers and necks of the vials were paraffined against water loss, and the vials, each containing about 12 cc of fluid and an equal amount of air, were stored away in near-darkness at a temperature that varied at different seasons from 15° C. to 25° C.

At the end of each year of the experiment the vials were uncorked for several hours, some air was bubbled through the fluid with a pipette, and a few drops of distilled water were added, if necessary. When the cysts were tested for viability, samples were removed at these times. Then the vials were stoppered and reparaffined as formerly.

At the end of the sixth year, the cysts were subjected to their first viability tests. With a pipette a hundred or more cysts from each of the 20 vials were transferred to a like number of finger bowls, each of which contained about 150 cc of vigorous hay-infusion culture of Paramecium. The transfer from old culture fluid to vigorous Paramecium culture constitutes a very reliable way of activating Didinium cysts (Mast, 1917a; Beers, 1935). After two days the finger bowls were examined for active didinia, and all gave positive results—all contained active specimens. Five control cultures (finger bowls containing only Paramecium culture) were always kept alongside the test cultures as a check upon possible contamination by wind-blown cysts of Didinium—an extremely remote eventuality—but these always resulted negatively. Microscopical examination of samples from

the vials disclosed a considerable number of empty cyst membranes, showing that many of the cysts had disintegrated, but, on the other hand, many still appeared to be living and showed clearly the resting, horseshoe-shaped macronucleus.

A year later the foregoing tests were repeated. Fifteen finger bowls gave positive results after two days; all gave positive results at the end of four days. Old cysts, I have often noted, sometimes require longer for activation.

At the end of the eighth year fourteen finger bowls, four days after the introduction of several hundred cysts into each, gave positive results. Repeated tests on cysts from the six remaining vials, until the supply was exhausted and the vials themselves were filled with infusion to activate cysts attached to their walls, gave only negative results, and these were discarded.

Nine years after encystment only two of the fourteen finger bowls gave positive results at the end of four days, although three of them contained active didinia after six days. Repetitions with larger numbers of cysts from the eleven remaining vials, until the supply in each was exhausted, resulted negatively. Thus, only three vials remained for continuing the experiment.

In October, 1936, ten years after encystment, what proved to be the final viability tests were made. In the first of the tests, using at least a third of the material remaining in each vial, the results were negative. Finally the entire contents of each of the vials were poured into three finger bowls and the vials themselves were filled with Paramecium culture. After six days one of the vials and its corresponding finger bowl each contained several dozen active, normal specimens of Didinium.

In conclusion, it is seen that some cysts of *Didinium nasutum* remain viable in hay infusion for at least ten years. Whether this is the maximum length of life of Didinium cysts, I am unable to say, but it is a fact that conditions appear to have been in general quite favorable within the vials. However, it may be argued that had the cysts received a better oxygen supply, their life would have been correspondingly prolonged.

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HATCHING PHEASANT CHICKS ON CHRISTMAS DAY[1]

Introduction

In two recent papers Bissonnette and Csech (1936a, b) report that ring-necked pheasants (*Phasianus colchicus torquatus*) were induced to lay fertile eggs beginning on January 15, 1936, instead of April 4 or 5, by subjecting one cock and four hens to added electric light after nightfall from December 16, 1935. Three of the hens averaged 105 eggs before June 29, and laying was continued till the hens were exhausted and finally died. The male did not seem to be damaged by the forced sexual activity. Similar treatment with a single pair of bob-white (*Colinus virginianus virginianus*) led to laying of fertile eggs from March 22 instead of the normal time, May 19 (Bissonnette and Csech, 1936a, c). Fertility of these precociously laid eggs was good in both breeds.

It seemed worth while to see if some of the pheasant chicks, hatched about the normal time from these early eggs, could be induced to lay in their first autumn; and, if so, what the percentage of fertility would be. Autumn hatches of eggs would demonstrate the possibility of almost continuous production of pheasants throughout the year, with early maturity of birds and reduced cost of keeping the birds before securing fertile eggs.

Material and Methods

On October 5, 1936, at 151 days of age, one ring-necked cock pheasant and five hens (*Phasianus colchicus torquatus*) hatched

[1] Aided by grants from the National Research Council, Committee for Research in Problems of Sex, 1936–37, and by cooperation of the Connecticut State Department of Fish and Game, in whose sanctuary at Farmington the experiments were carried out.

on May 7, 1936, were confined in the pen used in the previous experiment with pheasants (Bissonnette and Csech, 1936a, b). They were hatched from some of the eggs laid before the normal time as a result of winter night-lighting, but were not the earliest ones hatched. Those had been liberated in early autumn. These were, however, in full adult autumn plumage and much more nervous in temperament than their parents of the previous experiment. This may have been a factor in the failure of four of them to lay.

They were subjected to light from a 60-watt bulb for 3 hours per night (6 to 9 o'clock) for the first 10 days, 4, 5 and 6 hours for the second, third and fourth ten-day periods, respectively, and 7 hours per night thereafter till December 5, when the experimental lighting was stopped. Feeding and care were the same as for the rest of the pheasants at the sanctuary and the previous experiment.

OBSERVATIONS AND RESULTS

Within the 8 days before October 13, the cock assumed breeding plumage and head furnishings, began to give the mating call and to tread and copulate with at least two of the hens. It is not likely that he was producing sperms at that time, but his plumage and his behavior and that of the hens indicate that their secondary sexual characters and behavior were already responding to the increasing daily periods of light.

On November 8, one hen began to lay. She laid only 11 eggs, one of which was broken, before she stopped laying about November 28. None of the other hens laid any eggs before December 5, possibly because of frequent disturbance and nervous temperament.

On November 29, 10 eggs were set under a silky hen (the best breed to mother pheasant chicks). On Christmas day, at 26 days of incubation, three pheasant chicks hatched, another completed incubation but failed to chip the shell, and still another developed for about two weeks and was alive when the hen broke the egg shell.

This shows 50 per cent. fertility for these first ten eggs laid and a 30 per cent. hatch. So both this hen and the cock were completely active sexually at about 185 days of age, after 34 days of experimental lighting.

One of the chicks was stepped on by the hen before the first week was over. The other two are growing well and thriving with the hen in an improvised brooder at about 70° F.

Discussion

These chicks were hatched on Christmas day at 26 days of incubation from eggs laid between November 8 and 29 by a hen 185 days old, after just 34 days of experimental lighting. They were hatched just 232 days after their parents. This pair of pheasants would normally not have produced eggs before April 4, as judged by first laying in the colony at the sanctuary for two previous years. Under normal conditions a setting of 10 eggs from a single hen could not have been obtained before April 18 or 20 nor hatching before May 11.

The date of laying, therefore, was moved forward about 147 days (nearly 5 months) to 185 days of age from about 332 days, normal with the usual methods of game management, by only 34 days of lighting begun at 151 days of age. Hatching was moved forward about 137 days. Both sexes were activated synchronously, for 50 per cent. of the eggs were fertile.

This indicates that pheasants would likely lay normally in autumn or late summer but for the shortening days at these seasons, or if they were hatched early enough in spring to reach full growth and begin to lay before shortening days induce sexual regression in both sexes. Good early laying strains of domestic fowl do this, but not other strains. Night-lighting in autumn and winter, however, is used profitably to get eggs when prices are highest. That the ring-necked pheasant is capable of such performance with proper management is indicated by the results of this experiment.

It is probable that, if the earliest fertile eggs secured in January and February in the last winter's experiment (Bissonnette and Csech, 1936b) had been hatched at the earliest possible date, the young chicks would lay in late summer or that the November 8 date of the first egg could be greatly antedated. This is therefore not the earliest date at which fertile eggs may be secured or pheasant chicks hatched by proper, intelligent management. But, since artificially heated brooders and brooder houses are necessary to rear the chicks, the practical application of this method on a large scale for re-stocking hunting grounds is still of debatable advisability. It is a question of the increased cost of

heating brooders and brooder houses and lighting against the increased cost of feeding and caring for the parent stock for the longer time before normal laying, and the advantage of earlier chicks from some birds and later from others. It would give a continuous hatching and incubating period throughout autumn, winter and spring, with birds ready to be released at almost any time of year. That it can be done is no longer debatable.

SUMMARY

(1) One cock-pheasant and four hens, 151 days of age, were subjected to gradually increased night-lighting from October 5 to December 5.

(2) Breeding plumage and behavior was assumed within eight days and first egg laid by one hen on November 8.

(3) Eleven eggs were laid by one hen before cessation on November 28. The other four hens failed to lay, probably because of more nervous temperament and frequent disturbances.

(4) Of ten eggs set on November 29, three hatched at 26 days of incubation on Christmas day, one completed incubation but failed to hatch, and one developed till the egg was broken at two weeks. This is 50 per cent. fertility and 30 per cent. hatch.

(5) Both sexes therefore were completely activated at 185 days of age, with chicks hatched 138 days earlier than normally.

(6) It is possible to get pheasant eggs at any time in autumn, winter and spring and have chicks hatched and reared for liberation whenever desired if warm brooder houses are used.

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